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HAZARD CHARACTERIZATION FOR HUMAN HEALTH C8 EXPOSURE

CAS REGISTRY NO. 3825-26-1

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INTRODUCTION

This document is a Hazard Characterization of C8 for human health. C8 is also known as ammonium perfluorooctanoate (AFPO; CAS # 3825-26-1) and is the primary ingredient in FC-143 FLUORAD Brand Fluorochemical Surfactant. Within this document the chemical will be referred to as C8. However, it is acknowledged that many of the studies discussed actually tested the product FC143, which is a mixture of several straight-chain perfluorocarboxylic acids containing approximately 93.0-97.0% C8.

I. MAMMALIAN TOXICOLOGY

I.1. Acute Toxicity Studies

I.1.a. Acute Oral Toxicity

Numerous acute oral toxicity studies, in several species (rats, mice, guinea pigs, dogs), have been conducted with C8 (see Table I-1). The results of the various studies have been consistent in their results. Administration of a single dose of 12 mg/kg to 3 rats produced no clinical signs of toxicity. Studies demonstrate that newborn and older adult rats appear to be more sensitive than weanlings and young adults. Additionally, while mice and rats appear to be equally sensitive to the acute toxicity of C8, guinea pigs are more sensitive than mice or rats. In the rat, acute oral exposure generally results in enlarged livers, elevations of liver enzyme levels, gastrointestinal irritation, and weight loss. C8 is considered to have moderate acute oral toxicity.

In addition to the numerous studies listed below, several other studies were conducted which investigated the effects of C8 alone or on animals pre-exposed to other chemicals or drugs. Pre-treatment of rats with phenobarbital sodium or proadifen hydrochloride does not result in an alteration of the LD_{50} of C8 (478 mg/kg). Pre or post-dosing with Dowex® 1-X2-C1 Ion Exchange Resin at 1000 mg/kg reduced the mortality compared to rats dosed with C8 alone. A study was conducted to determine if pre-treatment with ethanol (a single dose of 60% or a 15% aqueous solution (v/v) in drinking water for 14 days) modifies the effects of C8 on liver weight. This study determined that pre-treatment with ethanol did not alter C8's effect on liver to body weight ratios.

Table I-1

SUMMARY OF ACUTE ORAL TOXICITY STUDIES WITH C8

Study Type	Species	#/sex/dose	Dose (mg/kg)	Vehicle	Results mg/kg	Reference
ALD	Rat	10 Males	200, 480, 670	Corn oil	480	HL-565-81
ALD	Rat	1 Male	1.5, 12, 40, 120, 200, 300, 450, 670, 1000, 1500, 2250	Water	670	HL-55-61
ALD	Rat	1 Male	1.0, 1.5, 2.3, 3.4, 5.1, 26, 40, 60, 77, 90, 120, 130, 170, 200, 300, 450, 670, 2250	Water	670	HL-128-68
LD_{50}	Rat	5	100, 215, 464, 1000, 2150	Acetone(49%) Corn oil (60%)	Males: 680 Females: 430	(Griffith and Long, 1980)
LD_{50}	Rat	10	200, 400, 450, 500, 670, 1000	Corn oil	Males: 470 Females: 482	HL-295-81
LD_{50} as a function of age	Rat					HL-788-82
		10 Weanling males	350, 400, 450, 525, 670, 710	Corn oil	573	
		10 Weanling females	350, 400, 450, 670		580	
		10 Young adult females	350, 425, 500, 670		453	
		10 Mature adult males	200, 240, 300, 350, 400, 500, 720		336	
		10 Mature adult females	225, 350, 400, 450, 670		343	
		10 Newborn males	130, 200, 240, 280, 330, 370		243	
		10 Newborn females	130, 180, 200, 220, 240, 280, 320		258	
LD_{50} castrated vs. intact adults	Rat					HL-600-81
		10 intact males	200, 480, 670	Corn oil	439	
		10 intact females	200, 480, 670		491	
		10 orchidectomized males	200, 480, 670		459	
		10 ovariectomized females	200, 480, 670		400	
LD_{50}	Rat	10 males	400, 500, 650	Corn oil	478	HL-567-81
LD_{50}	Rat				390	Hazleton Laboratory America, Inc. 2-6-87
LD_{50}	Mice	10	250, 500, 750, 1000, 2000, 4000	Corn oil	457	HL-329-81
Liver function	Dog	3 Males	450, 200	Not stated	Lethal at 450 mg/kg by 48 hours Elevated GPT and GOT which normalized within 1 week at 200 mg/kg	HL-123-65
LD_{50}	Guinea Pig	10	150, 200, 250, 300, 400, 670	Corn oil	Males: 178 Females : 217	HL-291-81

a). Weanling = 21-days old b). Young adult = 8-10 weeks old c). Mature adult = >10 weeks old d). Newborn = < 2 days old

I.1.b. Acute Dermal Toxicity

Acute dermal toxicity and irritation studies in rats and rabbits have been conducted with C8. C8 is considered to be mild - moderately irritating to the skin and moderately toxic by the dermal route of exposure. Rat skin showed less irritation than rabbit and in general the effects were more pronounced in males than in females. In addition to dermal irritation several clinical signs of toxicity were observed in both rats and rabbits in response to C8 exposure. These observations included body weight loss, wet and/or stained perineal area, cyanosis (rabbits only), diarrhea (rabbits only), lethargy (rabbits only), labored breathing (rabbits only), and chromodacryorrhea (rats at 7500 mg/kg)

Table I-2

SUMMARY OF ACUTE DERMAL TOXICITY/IRRITATION STUDIES WITH C8

Study Type	Species	#/sex/dose	Dose (mg/kg)	Results	Reference
LD_{50}	Rat	5	3000, 5000, 7500	Male LD_{50} = 6959 mg/kg Female LD_{50} = >7500 mg/kg	HL-659-79 (Kennedy, 1985)
Skin Absorption LD_{50}	Rat	5 Females	5000 and 7500	LD_{50}>7500 mg/kg Mild skin irritation	HL-682-80
Skin Absorption LD_{50}	Rat	5 Females	5000 and 7500	LD_{50}>7500 mg/kg Mild skin irritation	HL-682-80
LD_{50}	Rabbit	5 Males (2 at 7500)	1500, 3000, 5000, 7500	LD_{50} = 4278 mg/kg	HL-659-79 (Kennedy, 1985)
	Rabbit	4	100, 1000, 2000	Lethal 4/4 at 2000 3/4 at 1000 0/4 at 100	Riker Laboratories Report No. 09790AB0485
Skin Irritation	Rabbit	6	500 mg on intact and abraded sites	Non-irritating	(Griffith and Long, 1980)
Skin Irritation	Rabbit	6 Males	500 mg	Mild-moderate irritation at 24 hours Slight-moderate irritation at 48 hours	HL-636-79

I.1.c. Acute Ocular Toxicity

Eye irritation studies in rabbits have been conducted with C8. C8 is considered to be moderately irritating to the eye. Instillation of solid C8 into rabbit eyes produced moderate corneal opacity, iritis, and conjunctivitis. These ocular effects gradually receded over time. Prompt washing of the eye reduced the effects and provided a more rapid recovery. In addition to the eye irritation studies that have been conducted, rats

exposed to C8 during a 4-hour inhalation period exhibited corneal opacity and ulceration, which were microscopically evident 42 days post-exposure.

Table I-3

SUMMARY OF EYE IRRITATION STUDIES WITH C8

Species	#/sex/dose	Dose (mg)	Results	Reference
Rabbit	2 (1 unwashed, 1 washed)	38.3	Unwashed eye Moderate-severe corneal opacity Moderate iritis Moderate conjunctivitis At 21-28 days Corneal opacity Mild vascularization Washed eye Slight-moderate corneal opacity Slight-moderate conjunctivitis At 7 days Mild conjunctival redness At 14 days Normal	HL-635-79
Rabbit	6 unwashed 6 washed	100	Unwashed eye Moderate irritation Conjunctivitis Iritis Washed eye Conjunctivitis At 7 days 4/6 eyes were free of irritation	Biosearch, Inc. Report No. T1395 (Griffith and Long, 1980)

I.1.d. Acute Inhalation Toxicity

Acute inhalation toxicity studies in rats have been conducted with C8. Acute exposure to C8 by inhalation is considered to be highly toxic, with a 4-hour approximate lethal concentration (ALC) in rats of 0.8 mg/L. At concentrations of 2.2 mg/L C8 and higher, all rats died within 48 hours of exposure. At concentrations between 0.38 and 0.83 mg/L C8, rats experienced an initial weight loss following exposure and an increased liver-to-body weight ratio which returned to the high end of the normal range 42 days post-exposure. Additionally, all rats exposed to 0.81 mg/L C8 and higher showed corneal opacity and corrosion.

Table I-4

SUMMARY OF ACUTE INHALATION STUDIES WITH C8

Species	#/sex/dose	Concentration (mg/L)	Results	Reference
Rat	6 Males	4-hour exposure to: 0.38, 0.81, 0.83, 2.2, 4.8, 5.7	4-hour ALC = 0.8 mg/L LC_{50} = 0.98 mg/L	HL-160-69 (Kennedy, et al., 1986)
Rat	5	1-hour exposure to:18.6 mg/L	No deaths Eye and respiratory irritation	(Griffith and Long, 1980)

I.1.e. Acute Injection Toxicity

Acute toxicity of C8 when administered by intraperitoneal injection was assessed in mice (3M, 1979). The LD_{50} by intraperitoneal injection in mice is 192 mg/kg.

I.2. Subchronic Toxicity Studies

I.2.a. Subchronic Oral Toxicity

Numerous subchronic oral toxicity studies in several species (rats, mice, and monkeys), have been conducted with C8 (see Table I-5). The results of the various studies have been quite consistent in their results. Administration of C8 in the diet or by daily gastric intubation produced death at concentrations of 1000 ppm and higher for rats and mice and at 30 mg/kg/day for monkeys. The primary target organ for toxic responses in all species studied is the liver. C8 produces increased liver weights, increased liver enzyme activity, hepatocellular hypertrophy, and hepatic peroxisome proliferation.

In addition to the numerous studies listed below, other studies were conducted which investigated the mechanism of action of C8. These studies are summarized in Section III. Mechanisms of Action.

Table I-5

SUMMARY OF SUBCHRONIC ORAL TOXICITY STUDIES WITH C8

Study Type	Species	#/sex/dose	Concentration (ppm unless specified)	Results	Reference
14-day feeding	Mice	5	10, 30, 100, 300, 1000, 3000, 10000	100% mortality at ≥3000; deaths at 1000; increased liver weight/body weight ratio at ≥10	HL 560-81
14-day feeding	Mice	5	30, 300, 3000	100% mortality at 3000; deaths at 300; weight loss at ≥ 300 increased liver weight/body weight ratios at ≥ 30	HL12-82 (Kennedy, 1987)
21-day feeding	Mice	5	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30	Significantly increased liver weight at 30	HL 323-82 (Kennedy, 1987)
14-day feeding	Mice	5	30	Increased liver weight; when C8 was combined with an equal amount of nonadecafluorodecanoic acid, a similar effect was produced	HL 537-82
9-dose gavage	Mice	5	0.1, 1.0, 10 mg/kg	Weight loss, death in 10 mg/kg females, increased liver weight at 1 and 10 mg/kg	HL 138-83
	Rat	5	0.1, 1.0, 10 mg/kg	Weight loss, increased liver weight in 10 mg/kg males	
14-day feeding with a 2-week recovery period	Rat	6 Males	25% Teflon® with C8 as the dispersing agent	Slightly increased liver weights following the recovery period	HL 56-61
14-day feeding With a 56 day recovery period	Rat	5 Males	30, 300	Decreased body weights at 300; increased liver weights at the end of the feeding period at 30 and 300 and on recovery days 7 and 28 (300 ppm only; elevated blood fluoride levels out to recovery day 7 (final day tested)	HL 326-95

Table I-5 (Con't)

SUMMARY OF SUBCHRONIC ORAL TOXICITY STUDIES WITH C8

Study Type	Species	Sex (#/sex/dose)	Concentration (ppm unless specified)	Results	Reference
28-day feeding	Rat	5	30, 100, 300, 1000, 3000, 10000, 30000	100% mortality at ≥ 3000; decreased body weights at ≥ 1000 and 3000 for females; increased liver weight/body weight ratios at ≥ 30 for males and ≥ 300 for females	(Griffith and Long, 1980)
	Mice	5	30, 100, 300, 1000, 3000, 10000, 30000	100% mortality at ≥ 1000; deaths at ≥ 30; decreased body weights at ≥ 30; cyanosis and muscle weakness at ≥ 3000; increased liver weight/body weight ratios at ≥ 30; panlobular diffuse hypertrophy of hepatocytes	
90-day feeding	Rat	5	10, 30, 100, 300, 1000	Decreased body weights at ≥ 300; increased liver weights at ≥ 300; panlobular diffuse hypertrophy of hepatocytes at ≥ 1000 with males more affected than females; serum fluoride concentration increased 75 to 226 fold with higher concentrations observed in males	
90-day gavage	Monkey	2	3, 10, 30, 100 mg/kg/day	100% mortality at ≥ 100; deaths at ≥ 30 (females only); decreased body weights at ≥ 30; no signs of toxicity at 3 mg/kg/day; dose dependent increases in serum and liver fluorine levels (no apparent sex difference)	
90-day feeding with an 8-week recovery period	Rat	55 Males	1, 10, 30, 100	Reduced body weigh at 100; increased palmitoyl CoA oxidase activity at ≥ 30 and transient increases at 10; palmitoyl CoA oxidase activity returned to normal after the 8 weeks of recovery; increased liver weights and hepatocellular hypertrophy at ≥ 10 which was reversible following the recovery period. Serum estradiol, testosterone and lutienizing hormone levels were not affected by dietary exposure to C8, while estradiol levels were slightly elevated at 100 ppm at week 5. The NOAEL = 100 ppm; the NOEL = 1 ppm	(Perkins, 1992)

I.2.b. Subchronic Inhalation Toxicity

Similar to the oral toxicity studies, inhalation exposure to C8 produces reduced body weight, increased liver weights, increases in plasma enzymes indicative of liver injury, and pathological lesions in the liver. Measurement of the blood fluoride levels (indicative of the presence of C8) determined that the blood half-life of C8 in the rat is 5-7 days following inhalation exposure.

Table I-6

SUMMARY OF SUBCHRONIC INHALATION TOXICITY STUDIES WITH C8 IN THE RAT

Study Type	Sex (#/sex/dose)	Concentration	Results (mg/kg)	Reference
10 exposure with a 42-day recovery	20	11, 83 mg/m^3 for 6 hours/day	Dose related decrease in body weight, suppression of body weight maintained during the 42-day recovery period at 83; increased plasma enzymes indicative of liver injury present up to 28 days following the last exposure; granular degeneration of hepatocytes; increased liver weights, no ocular effects were observed. The liver effects were not observed after 14, 32, or 42 days of recovery.	HL 253-79
10 exposure with an 84-day recovery	24	1, 8, 84 mg/m^3 for 6 hours/day	Deaths at 84; increased lung, liver and testes weights, no ocular effects observed; increased plasma enzymes indicative of liver injury; increased liver weights at $\geq$ 8 mg/m^3; panlobular and centrilobular hepatocellular hypertrophy and necrosis. The liver effects were reversible following a 28-day recovery period. Dose related presence of C8 in the blood, which decreased with time during the recovery period but was still detectable after 84 days of recovery. NOAEL = 1 mg/m^3, although 13 ppm organofluoride was detected immediately following exposure to 1 mg/m^3	HL 205-81 (Kennedy, Hall et al., 1986)

I.2.c. Subchronic Dermal Toxicity

The subchronic dermal toxicity of C8 has been studied in the rat and rabbit (see Table I-7). Similar to the oral toxicity studies, dermal exposure to C8 produces reduced body weight, increased liver weights, increases in plasma enzymes indicative of liver injury and lesions in the liver. Measurement of blood fluoride levels (indicative of the presence of C8) determined that the blood half-life of C8 in the rat is 5-7 days following dermal exposure. A comparison of the dermal exposure studies to the feeding studies leads to the conclusion that the rates of absorption of C8 by these two routes are not significantly different.

Table I-6

SUMMARY OF SUBCHRONIC INHALATION TOXICITY STUDIES WITH C8 IN THE RAT

Study Type	Species (#/sex/dose)	Concentration (mg/kg)	Results (mg/kg)	Reference
10 dose with an 84 day recovery	15 Male Rats	20, 200, 2000 for 6 hours/day, 5 days/week	Skin irritation at ≥ 200; reversible reduction in body weight at ≥ 200; increased plasma enzymes indicative of liver injury; increased liver weights at ≥ 20; hepatocellular hypertrophy and necrosis at 20 ; no ocular effects observed The liver effects were generally reversible following a 42-day recovery period at ≤ 200. Dose related presence of C8 in the blood, which decreased with time during the recovery period but was still detectable after 42 days of recovery.	HL 589-80 (Kennedy, 1985)
Range-finder	4 Rabbits (sex not specified)	100, 1000, 2000	Lethal to 4 of 4 at 2000, 3 of 4 at 1000, 0 of 4 at 100	Riker Laboratories, Report 09790AB0485, March 15, 1981
10 exposure with a 14 day recovery	10 Rabbits	100 for 6 hours/day, 5 days/week	Reversible reduction in body weight; Blood fluorine levels were 5.4, 6.8, 4.6 ppm for males and 10.1, 12.1, and 3.5 for females at 7, 14, and 28 days of the study, respectively.	

I.3. Developmental Toxicity

Developmental toxicity studies have been conducted in rats and rabbits (See Table I-7). The original developmental toxicity study in rats indicated that C8 might be a teratogen in rats. However, because the results were questionable, additional studies were conducted to clarify the result. The additional studies did not confirm the original result. Overall, C8 is not considered to be uniquely hazardous to the conceptus.

The two areas of question were apparent lens abnormalities and skeletal alterations. In the original study, the lens alterations consisted of the following: large lens cleft, dark streak running ½ to ¾ of the way through the lens; or disorganized lens fibers. In the subsequent studies, the lens alterations were determined to be an artifact created in the lens during freehand sectioning. Processing Bouin's-fixed fetal heads that were trimmed on either side of the orbit, instead of through the center of the eye, essentially eliminated this artifact. Examination of the eyes of offspring using focal illumination, indirect ophthalmoscopy, and slitlamp microscopy were also used and did not detect any C8-related eye alterations. The skeletal alterations included ossification sites on the first lumbar vertebrae in rats and 13 ribs in rabbits. Both of these alterations are considered to represent stress-related changes indirectly related to C8-administration.

Table I-7

SUMMARY OF DEVELOPMENTAL/REPRODUCTIVE TOXICITY STUDIES WITH C8[a]

Species (#/dose)	Concentration	Results mg/kg	Reference
Rats (# not specified)	25, 50, 75, 100, 150 mg/kg by gavage	Reduced maternal body weight gain and clinical signs of toxicity at 150; eye abnormalities at 25 and 150	3M Report M-601 (1981)
25 Rats[b]	100 mg/kg by gavage	Maternal deaths, decreased maternal body weight gain; no developmental toxicity or abnormalities observed.	HL 1-82 (Staples, et al., 1984)
12 Rats[c]	100 mg/kg by gavage	Maternal deaths, decreased maternal body weight gain; no alterations in postpartum viability, growth rate, or development. No ocular effects observed.	
Rats (# not specified)	0.05, 1.5, 50, 150 mg/kg by gavage	Maternal deaths at 150; C8 was not embryotoxic, no abnormal gross findings, no malformations[d]. Fetal lens findings were observed in all groups. Determined to be a processing artifact. No effect on ovaries, reproductive tract, male/female ratio, implantation sites, corpora lutea, or fetal weights.	3M Report 0681TR0110, 1981
Rats	0.14, 1.2, 9.9, 21 mg/m^3 by inhalation	Maternal deaths at 21; overt maternal toxicity at 9.9; No teratogenic effects were observed in any of the exposed groups; embryo-fetal toxicity was observed at 21; processing artifacts were observed on lens'.	HL 881-81 (Staples, et al., 1984)
18 Rabbits	1.5, 5, 50 mg/kg by gavage	Reduced maternal body weight gain at 50, C8 was not embryotoxic or teratogenic[e]	3M Product Toxicity Sheet, May 24, 1996

a. Pregnant rats were dosed by gavage on days 6-15 of pregnancy. Pregnant rabbits were dosed by gavage on days 6-18 of pregnancy.
b. Sacrificed on Day 21 of gestation.
c. Pups sacrificed on day 35 postpartum.
d. A significantly higher incidence of the skeletal finding "one sternabrae missing", occurred in the high-dose group. This was a minor skeletal aberration and was not considered a malformation in this study. Furthermore, the incidence of this finding did not differ from the control group or the 3 lower-level treatment groups. The incidences of skeletal findings associated with delayed ossification and rib aberrations were not different among the treatment groups and controls.
e. There was a statistically significant increase in the incidence of 13 ribs in the high dose group and 13 ribs spurred in the mid-dose group. While the findings are significantly greater in the treated animals than in the controls, they are not considered to be teratogenic changes or malformations, rather they are considered to represent stress-related changes to compound administration.

I.4. Reproductive Toxicity

No information is available on the reproductive toxicity of C8

I.5. Mutagenicity

It has been demonstrated that C8 is not mutagenic in a variety of mutagenicity tests (See Table I-9).

Table I-9

SUMMARY OF MUTAGENICITY STUDIES WITH C8 IN THE RAT

Study Type	Study Description	Results	Reference
Mutagenicity assay	Assayed in *S. Typhimurium* (TA1535, TA1537, TA1538, and TA100) and *S. cerevisiae* D4 yeast, with and without metabolic activation.	Negative	Litton Bionetics; LBI Project 20838, Feb. 1, 1978 (Griffith and Long, 1980)
In vivo mouse micronucleus	3 mice/sex were dosed with 200, 400, 600, 800, and 1000 mg/kg and bone marrow was evaluated at 24, 48 and 72 hours after dosing.	Negative	Corning Hazleton, 17388-0-455, May 16, 1996
Chromosomal aberration	Assayed for ability to induce chromosomal aberrations in CHO cells with and without metabolic activation.	Negative	Corning Hazleton, 17388-0-437, April 25, 1996
Mammalian cell transformation assay	Assayed for cell transformation potential and cytotoxicity in C3H 10T1/2 colony cells.	LD = 50 g/mL; low cytotoxicity No evidence of cell transformation	University of Minnesota Environ. Path Lab, T2942, April 9, 1981

I.6. Chronic Toxicity and Oncogenicity

The chronic toxicity and oncogenicity of C8 has been investigated in two 2-year feeding studies in rats (see Table I-10).

Table I-10

SUMMARY OF CHRONIC TOXICITY AND ONCOGENICITY STUDIES WITH C8 IN RATS

#/sex/dose	Concentration (mean daily intake, mg/kg/day)	Results	Reference
50	0, 30, 300 ppm (0, 1.5, and 15 mg/kg/day)	Decreased body weight gain and food consumption, increased ataxia. Decreased RBC counts, hemoglobin, and hematocrit values. Increased liver weights, liver cell hypertrophy, degeneration and necrosis. Not considered to be carcinogenic.	Riker Laboratory 0281CR001 (April 1981–May 1983)
156 Males	0, 0-pair-fed, 300 ppm	Decreased body weight gain and food consumption. Increased estradiol levels. Increased incidence of liver, Leydig cell and pancreatic acinar cell adenomas.	(Cook, et al., 1994) DuPont MR-5686

In the original study, in-life findings consisted of a dose dependent decrease in mean body weight gain and increase in food consumption in males, and a slight treatment-related increase in the incidence of ataxia in females. No increase in mortality was observed. C8-related hematologic alteration included decreased red blood cell counts, hemoglobin and hematocrit values observed at various times throughout the 2-year test period. However, the decreases in erythrocyte counts were observed early in the study and did not progress into generalized anemia. Histopathologically, C8-associated alterations were observed in the liver. These changes were characterized by increased liver weights, hypertrophy, hepatocellular degeneration, and necrosis. As with the erythrocyte counts, the hepatic alterations were observed early in the study and showed little progression over the remainder of the 2-year study. The incidence of tumors was relatively low, and the types of neoplasms found were not different from the tumor profiles commonly observed in geriatric rats. Hepatocellular tumors were slightly increased in the 300 ppm males, however, not to the extent that would be expected considering the morphological evidence of hepatocellular stimulation observed at the 1-year necropsy. The incidence of testicular Leydig cell adenomas (0/50, 3/50, and 7/50 at 0, 30, and 300 ppm, respectively) was suggestive of a compound-related effect. However, because the incidence was within the historical control range, it was not considered to be a compound-related effect. Based on the tumor incidence, types of tumors, time of tumor appearance, and the survival rate at the 2 year time point, the overall conclusion was that C8 was not carcinogenic in the rat (Riker Laboratory, 0281CR0012). However, in this original study, some of the pathological findings were equivocal (liver and Leydig cell tumors), even when evaluated by an outside laboratory, and therefore a second 2-year study was conducted to clarify some of these findings.

The second study included many mechanistic endpoints to help determine the mechanism of tumor formation (DuPont MR-5686, Cook, et al., 1994). In addition to the *ad libitum* control, a second control was pair-fed to the C8 group. Peroxisome proliferation (ß-oxidation activity) and cell proliferation (BrdU, 6-day osmotic pumps)

were measured in the liver and testis. Serum hormone levels (testosterone, estradiol, lutienizing hormone (LH), follicle stimulating hormone (FSH) and prolactin) were also measured. Interim sacrifices were performed at 3-month intervals as well as at 1 month. Increased relative liver weights were observed in the C8-treated rats. Hepatic ß-oxidation activity was also increased in the C8-treated rats at all time points. In contrast, hepatic cell proliferation was not significantly increased in the C8-treated group. C8 did not significantly alter the rate of Leydig cell ß-oxidation or Leydig cell proliferation. Moreover, the rate of ß-oxidation in Leydig cells was approximately 20-times less than the rate of hepatic ß-oxidation, irrespective of treatment. Serum testosterone, FSH, prolactin, and LH levels were unchanged in the C8-treated rats when compared to the controls. There were, however, significant increases in serum estradiol levels in the C8-treated rats at 1, 3, 6, 9, 12, 15, 18 and 21 months. Histopathological evaluation revealed compound-related increases in liver, Leydig cell, and pancreatic acinar cell tumors in C8-treated rats. Based on the data, the Leydig cell tumors appear to be due to the combination of elevated estradiol levels and reduced prolactin levels. The pancreatic acinar cell tumors are related to an increase in serum cholecystokinin (CCK) levels.

II. METABOLISM

Numerous studies have been conducted investigating the excretion and disposition of C8 in various species. Additionally, studies have been conducted with exposed workers at a manufacturing plant which produces C8. Sex and species differences have been noted, whereas reproductive status in females did not have an effect on excretion or disposition in rats. Rabbits (both sexes), female rats, and male hamsters rapidly excrete C8, while male rats and female hamsters excrete C8 more slowly. Mice (both sexes) excrete C8 even more slowly. C8 also has a long ½-life in humans. Measurement of C8 blood levels in an exposed worker showed that the ½-life in men is greater than 1.5 years.

II.1. Animal Studies

The excretion and disposition of C8 has been investigated in rats, mice, hamsters and rabbits. Studies have also investigated the influence of route of exposure. These studies are summarized below.

II.1.a. Male and female rats were administered radiolabeled C8 by intravenous injection. Females excreted essentially 100% of the administered dose by 24 hours, while males had excreted only 20% of the administered dose. Radioactive tissue residues were not detectable after 17 days in the females, while at 36 days males had 2.8% of the ^{14}C in the liver, 1.1% in the plasma and lower but detectable levels in other organs (Riker Laboratory drug Metabolism Report 1-20 (1980)).

II.1.b. Sex differences in the excretion and disposition of radiolabeled C8 were observed in a study of rats, mice, hamsters, and rabbits. Male and female animals of each species were dosed by gavage with 10 mg/kg C8, and urine and feces were collected at 24, 48, 72, 96, and 120 hours post-dosing. Animals were then sacrificed, and blood and

tissues were analyzed. The urine and feces of rabbits was also collected at 144 and 168 hours post-dosing, and rabbits were sacrificed at 168 hours.

The female rat and male hamster had excreted over 99% of the administered dose at the time of sacrifice. The male rat and the female hamster had excreted 39 and 60% of the administered dose, respectively, at the time of sacrifice. Both sexes of rabbits excreted the C8 as rapidly and completely as the female rat and male hamster. The male and female mice retained substantial amounts of the total administered radioactivity in their tissues at the time of sacrifice, only excreting 21% of the administered dose at 120 hours post-dosing (HL 62-82)

II.1.c. Cholestyramine, a non-absorbable anion-exchange resin, was demonstrated to protect rats from the acute lethal effect of C8 when administered within 2 hours of C8 dosing (HL 828-81).

A second study was conducted to investigate the effect of cholestyramine on the elimination of ^{14}C-C8 (10 mg/kg by gavage) from rats and mice (HL 405-82). Adult male rats and mice were given cholestyramine (1000 mg/kg by gavage) 24 hours after dosing with ^{14}C-C8. The cholestyramine did not enhance the elimination of C8 via the feces, urine, or exhaled air. Similarly, Dowex® Ion Exchange Resin was also able to reduce the acute lethal effect of C8. When rats and mice were given Dowex® resin 24 hours after dosing with C8 no signs of enhanced elimination of C8, via the feces, urine or exhaled air, were observed (HL 405-82).

To further investigate the use of cholestyramine to enhance C8 elimination, a third study was conducted in rats. In this study, rats were dosed with ^{14}C-C8 (13.3 mg/kg, by iv.) and then were fed diets containing 4% cholestyramine for 14 days. The cholestyramine increased the elimination of C8 via the feces by 9.8 fold and decreased the concentration of C8 found in the liver, plasma, and red blood cells (Johnson, et al., 1984).

II.1.d. A series of experiments was conducted to evaluate the uptake and clearance of C8 from the blood of male and female (pregnant and non-pregnant) rats following oral exposure, and inhalation exposure.

The uptake and clearance of C8 from the blood of female rats following a single oral dose was rapid, with peak reached 1-2 hours post-treatment and virtual total clearance by 24 hours. A dose-response was demonstrated with no apparent changes in blood C8 levels following multiple oral dosing. The slower clearance rate in male rats was demonstrated following a single oral dose. The same general statements apply following inhalation exposure. A single 6-hour inhalation exposure resulted in: peak blood levels within 1 hour after cessation of exposure; the material rapidly cleared from the blood; the number of exposures did not affect blood levels; and male rats cleared the compound much more slowly. Pregnant and non-pregnant rats showed similar C8 blood

levels following either oral or inhalation exposure (HL 593-91). Specifics of the experiments are summarized below.

II.1.e.1. Oral administration-Blood levels of C8 as a function of time post-dosing (female rats)

C8 levels of 14 ppm were seen 15 minutes following administration of C8. These levels peaked at 30 ppm at 1-2 hours, dropped to 26 ppm by 8 hours, and to 0.7 and 0.045 ppm at 24 and 168 hours, respectively. C8 is absorbed and rapidly cleared from the blood of female rats given a single oral dose.

II.1.e.2. Oral administration-Blood levels of C8 as a function of dose (female rats)

C8 levels 30 minutes following administration of 2.5 – 150 mg/kg ranged from 3 –162 ppm. The same dose response was observed at 24 hours with blood levels ranging from 0.12 - 18 ppm. The response was linear. The level of C8 in the blood is directly related to the amount of C8 administered.

II.1.e.3. Oral administration-Blood levels of C8 as a function of number of doses (female rats)

Blood levels in female rats given 1 versus 11 doses of C8 were not considerably different. Concentrations at 15 minutes following administration were 14 and 17 ppm for 1 and 11 doses, respectively. At 30 minutes C8 concentrations were 16 and 25 ppm; at 8 hours 26 and 13 ppm; at 24 hours, 0.7 and 0.8 ppm; and at 168 hours, 0.045 and 0.10 ppm for 1 and 11 doses, respectively. C8 does not appear to accumulate in the blood of female rats following repeated oral administration. The number of treatments does not appear to influence the C8 blood level.

II.1.e.4. Oral administration-Blood levels of C8 following a single 25 mg/kg dose (male and female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Male Rats	Female Rats
½	23	16
8	63	26
24	50	0.7
168	23	0.045

C8 is retained in the blood of male rats to a greater extent than female rats.

II.1.e.5. Inhalation exposure-Blood levels of C8 as a function of time post-exposure (female rats)

C8 levels of 96 ppm were observed 15 minutes following a single 6-hour exposure to 10 mg C8/m^3. The level was maintained through 1 hour, fell to approximately 70 ppm at 8 hours, 52 ppm at 24 hours, and dropped to 0.39 ppm at 168

hours post-exposure. This same general pattern was observed in rats exposed to either 0.1 or 1 mg/m^3. The lag phase seen following oral exposure was not observed here due to blood sampling following a 6-hour inhalation exposure (rather than a single dose at a given time). C8 is absorbed and rapidly cleared from the blood of female rats following a single inhalation exposure.

II.1.e.6. Inhalation exposure -Blood levels of C8 as a function of dose (female rats)

Time following single inhalation exposure dose (hours)	Blood Levels of C8 (ppm) Following exposure to C8 at		
	0.1 mg/m^3	1 mg/m^3	10 mg/m^3
½	2	7	109
2	2	17	69
8	0.85	4	71
24	0.14	0.56	52

The response is linear at 30 minutes. C8 blood levels are directly related to the amount of C8 inhaled. At the high concentration used, the clearance rate is somewhat slower than observed at the lower levels. This suggests massive overloads in the clearance system.

II.1.e.7. Inhalation exposure -Blood levels of C8 following a single 6 hour exposure to 10 mg/m^3 (male and female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Male Rats	Female Rats
½	137	109
2	157	69
8	182	71
24	147	52

C8 is retained in the blood of male rats to a greater extent than female rats following inhalation exposure.

II.1.e.8. Oral administration-Blood levels of C8 following a single 25 mg/kg dose (pregnant and non-pregnant female rats)

Time following single oral dose (hours)	Blood Levels of C8 (ppm)	
	Pregnant Rats	Non-pregnant Rats
½	16	10
2	33	39
8	26	31

C8 clearance following oral dosing is similar in pregnant and non-pregnant female rats.

II.1e.9. Oral and Inhalation exposure -Blood levels of C8 as a function of number of exposure concentration (pregnant female rats)

Time following single oral dose of 25 mg/kg	Blood Levels of C8 (ppm) Following		
(hours)	1 exposure	6 exposures	10 exposures
½	18	12	12
2	39	37	15
8	31	nd[a]	11
24	2	nd	1

Time following single inhalation exposure to 10 mg/m^3	Blood Levels of C8 (ppm) Following	
(hours)	1 exposure	10 exposures
½	77	53
2	90	nd

a. nd = not done

When comparing the C8 levels seen at 2 and 8 hours, following 10 consecutive oral doses, there appears to be a lowering of the C8 blood levels. Blood levels following 1 or 10 consecutive inhalation exposures (6 hours/day) were not different. C8 does not appear to accumulate in the blood of pregnant rats following repeated oral or inhalation exposures.

II1.f. The ability of ^{14}C-C8 to transfer through the placenta was investigated in rats (HL 61-82). A single dose of 10 mg/kg ^{14}C-C8 was administered to pregnant rats on the 19th day of pregnancy. Maternal blood and placental levels of ^{14}C-C8 increased between 2 and 4 hours post-dosing, and decreased between 4 and 8 hours post-dosing.

Time following single oral dose of 10 mg/kg	Levels of C8	
	Maternal	Fetal
(hours)	(µg equivalents/mL blood)	(µg equivalents/mL tissue)
2	12	0.7
4	20	3
8	12	3

II.2. Human Exposure

Determinations of organic fluorine blood levels in workers exposed to C8 in an industrial environment were performed. Approximately 90% of the organic fluorine was composed of the C8 anion. The highest levels were found in workers with the longest work history in fluorochemical production. The majority of the values remained at approximately the same level throughout the 2 ½ year monitoring period. Monitoring of C8 blood levels of a worker who was removed from the fluorochemical production site due to high C8 blood levels (70 ppm) suggests that fluorochemicals are very slowly

eliminated. From this limited data it is hypothesized that the ½-life of C8 is 1.5 years in men (Ubel, et al., 1980).

Group	Number analyzed	Blood Organic Fluorine Levels (ppm)
Normal human sera	from published literature	0.01-0.13
Industrial controls	4	0.01-0.08
Laboratory personnel (>20 years exposure)	8	0.04-2.00
Plant workers	49	1.00-71.00

III. MECHANISMS OF ACTION

C8 is not metabolized in rats. C8 produces hepatomegaly, induces hepatic peroxisomes in mice and rats, and has been shown to produce hepatic, Leydig cell, and pancreatic acinar tumors in a 2-year feeding study in rats. The male rat is more susceptible to the toxic effects of C8 than the female rat, presumably due to the longer ½-life in males. Short-term studies have been conducted investigating the mechanisms of action responsible for the various effects.

III.1. Investigation of C8s' Effect on the Liver.

III.1.a. Because C8 had been shown to induce a striking hepatomegaly in rats, a study was conducted to investigate the hepatic biochemical and morphological changes associated with C8-induced hepatomegaly in rats (Pastoor, et al., 1987). In this study male rats were dosed daily for 1, 3, or 7 days with 50 mg C8/kg body weight by intragastric intubation. The total cytochrome P450 content and activity of benzphetamine *N*-demethylase was increased in the livers of C8-treated rats, indicating the proliferation of smooth endoplasmic reticulum. In contrast, the soluble, cytoplasmic enzymes, glutathione *S*-transferase and UDP-glucuronyltransferase, were unaffected. Carnitine acetyltransferase activity was disproportionately increased relative to carnitine palmitoyl transferase activity, confirming the predominant proliferation of peroxisomes versus mitochondria. Electron microscopy confirmed the proliferative response of the endoplasmic reticulum, peroxisomes, and microsomes in the livers of the C8-treated rats. This study also demonstrated that C8 does not possess hypolipidemic activity.

III.1.b. C8 increased serum estradiol concentrations in 2-week gavage studies, and feeding studies at various time points up to 2 years. This was accompanied by increases in liver weights, and hepatic β-oxidation activity (Cook, et al., 1992; Cook, et al., 1994). Since peroxisome proliferators induce both β-oxidation activity and cytochrome P450 enzymes, an investigation was conducted to determine if C8 increases serum estradiol levels by stimulating aromatase activity (Liu, et al., 1996a). Fourteen days of treatment with up to 40 mg C8/kg/day produced dose-dependent increases in liver weights, serum estradiol, and hepatic aromatase activity. A significant linear correlation was established between estradiol and hepatic aromatase activity. *In vitro* experiments using cultured hepatocytes suggest that the increase in serum estradiol is at least partly

due to a direct effect on the liver to increase synthesis of estradiol through induction of aromatase cytochrome P450 in the endoplasmic reticulum.

III.2. Investigation of C8s' Effect on Testicular Leydig Cells

Because C8 produced an increased incidence of testicular Leydig cell tumors in a 2-year feeding study in rats, and because C8 was negative in short-term tests for genotoxicity, a non-genotoxic (hormonal-mediated) mechanism for tumor formation was investigated. The studies summarized below support a hormonally-mediated mechanism of Leydig cell tumorigenesis: C8 produces an increase in hepatic aromatase activity, which elevates serum estradiol concentrations, which in turn modulates growth factors in the testis, which results in tumor formation.

III.2.a. Fourteen days of treatment with up to 50 mg C8/kg/day produced dose-dependent increases in hepatic β-oxidation activity, and serum concentrations of estradiol, and decreases in serum testosterone concentrations, body weights, and relative accessory sex organ weights in male rats (Cook, et al., 1992). Challenge experiments, using human chorionic gonadotropin (hCG), gondaotropin-releasing hormone (GnrH), or naloxone challenges, suggest that the decrease in testosterone may be due to a lesion at the level of the testes, due to a decrease in the conversion of 17α-hydroxyprogesterone to androstenedione.

III.2.b. Using *in vitro, in vivo* and *ex vivo* studies, C8 was examined for its ability to directly affect Leydig cells *in vitro* using isolated Leydig cells from untreated rats, and *ex vivo* using Leydig cells isolated from C8-treated rats. Additionally, the ability of C8 to affect testicular interstitial fluid hormone levels and induce aromatase activity was investigated (Biegel, et al., 1995). The *in vitro* studies demonstrated that C8 directly inhibits testosterone production, while the *ex vivo* studies demonstrated that this inhibition is reversible. In the *in vivo* study, serum and testicular interstitial fluid estradiol were increased and testicular interstitial fluid transforming growth factor α were increased. Additionally, hepatic aromatase activity was increased while aromatase activity levels were not affected in the testis, muscle, or fat. These data suggest that the increases in estradiol levels are primarily due to increases in aromatase activity.

III.2.c. Previous studies with C8 showed a direct effect on Leydig cells to alter steriodogenesis. It was therefore proposed that peroxisome proliferators, in general, may directly affect Leydig cell function to produce Leydig cell tumors. A study investigating whether several peroxisome proliferators (including C8), directly affect Leydig cell function *in vitro* was conducted. This study showed that peroxisome proliferators, as a class of compounds, directly modify the steroidogenic function of Leydig cells *in vitro*. This also suggests that compounds which directly affect Leydig cell function *in vitro* may also induce Leydig cell tumors *in vivo* (Liu, et al., 1996b).

III.3. Investigation of C8s' Effect on the Pancreas

Several peroxisome proliferators have been shown to produce pancreatic acinar cell hyperplasia/adenocarcinomas in 2-year feeding studies, including C8. Therefore, *in vitro* and *in vivo* investigations of C8's (*in vitro* only) and Wyeth-14, 643's (a model peroxisome proliferator) mechanism of tumorigenesis in the pancreas were conducted. These mechanisms include cholecystokinin receptor agonism (CCK_A) trypsin inhibition, alterations in gut fat content, cholestasis and altered bile flow/composition. All of these mechanisms enhance pancreatic growth either by binding to the CCK_A receptor or by increasing plasma CCK levels. C8 did not bind directly to the CCK_A receptor and it failed to inhibit trypsin, a common mechanism for increasing plasma CCK levels. *In vivo* studies with Wyeth-14, 643 suggest that these peroxisome proliferators produce pancreatic tumors by cholestasis, which may be responsible for the decrease in bile acid output which contributes to the increase in plasma CCK levels. Therefore, for Wyeth-14, 643 (and perhaps C8), the pancreatic tumors may be secondary to hepatic cholestasis (Obourn, et al., 1997).

IV. CLINICAL REPORTS OF HUMAN EXPOSURE

IV.1.a. Health screening examinations were offered to employees of a 3M plant that produced C8, as well as other fluorochemicals. No health problems related to exposure were encountered among those examined. Additionally, no relationship was observed between deviations from normal laboratory test results and blood levels of organic fluorine (the liver enzyme SGGT was the most frequently encountered test result exceeding the normal range. C8 exposure levels ranged from 0.03 to 7.6 mg/m^3 (Ubel, et al., 1980).

IV.1.b. A study was made of Washington Works employees potentially exposed to C8. Results of blood chemistry testing (SGOT, LDH, AP, and bilirubin) indicated no conclusive evidence of an occupationally related health problem among workers exposed to C8 (Fayerweather, 1981).

IV.1.c. Although C8 is the major organofluorine compound found in humans, little information is available concerning human responses to C8 exposure. Therefore, a study was conducted among 115 workers exposed to C8 occupationally (serum fluorine levels varied between 0 and 26 ppm, with a mean of 3.3). In an examination of the cross-sectional associations between C8 and hepatic enzymes, lipoproteins, and cholesterol, there was no significant clinical hepatic toxicity of the C8 levels observed in this study (Gilliland and Mandel, 1996). Serum C8 levels were positively associated with estradiol and negatively associated with free testosterone and not associated with luteinizing hormone. The negative association between testosterone and C8 was stronger in older men. Thyroid stimulating hormone and C8 were positively associated. Prolactin and C8 were positively associated in moderate drinkers. The effect of adiposity on serum glutamyl oxaloacetic acid and glutamyl pyruvic transaminase decreased as C8 increased. The induction of gamma glutamyl transferase by alcohol was decreased as C8 increased. The effect of alcohol on HDL was reduced as C8 increased. A positive association

between hemoglobin, mean cellular volume, and leukocyte counts with C8 was observed. These results suggest that C8 affects male reproductive hormones and that the liver is not a significant site of toxicity in humans at the C8 levels observed in this study. However, C8 appears to modify hepatic and immune responses to xenobiotics (Gilliland and Mandel, 1993).

V. EPIDEMIOLOGY

V.1.a. A retrospective cohort mortality study was made of employees at a 3M plant where C8 and other fluorocompounds are manufactured. Records on 4218 employees were reviewed. Only those who worked for 6 months or more (3688 workers) were included in the mortality follow-up. Of the 180 known deaths, 177 death certificates were obtained. Overall the number of deaths was significantly less than expected. The observed-to-expected ratio for cancer deaths was 1.0 (Ubel, et al., 1980).

V.1.b. In a retrospective cohort mortality study, a relationship between mortality and employment at a plant where C8 and other fluorocompounds are manufactured were investigated (Gilliland and Mandel, 1993). The cohort consisted of 2788 male and 749 female workers employed between 1947 and 1983. The all-causes standardized mortality rate (SMR) was 0.75 for males and 0.77 for females. There was no significantly increased cause-specific SMR for men or women. The SMRs for prostate cancer were 2.03 in the exposed group and 0.58 in the not-exposed group. In the exposed group there were 4 observed and 2 expected deaths from prostate cancer. Among men, 10 years of employment in C8 production was associated with a significant 3-fold increase in prostate cancer mortality when compared to no employment in production. Given the small number of prostate cancer deaths and the natural history of the disease, the association between production work and prostate cancer must be viewed as hypothesis generating and not over interpreted. If the prostate cancer mortality excess is related to C8, the results of this study and other clinical studies suggest that C8 may increase prostate cancer mortality through endocrine alterations.

VI. DISCUSSION OF ENDPOINTS

VI.1. Discussion of Target Organs

The primary target organ for C8-induced toxicity is the liver in mice, rats, and dogs, regardless of route of exposure. The hepatotoxicity manifests as increased liver weights, hepatocellular hypertrophy, liver degeneration, increases in liver enzymes, necrosis of the liver, and induction of peroxisomes (rats and mice only). Many of these effects were demonstrated to be reversible when animals were provided with a recovery period. Evidence of hepatotoxicity was not evident in studies in monkeys or humans.

In contrast with the rodent, the target organs in the monkey were the

gastrointestinal tract and the reticuloendothelial system (Griffith and Long, 1980). While the liver does not appear to be a primary target organ in humans, exposure to C8 appears to modify the hepatic and immune response to xenobiotics (Gilliland and Mandel, 1996).

VI.2. Discussion of Differences in Species-Specific Sensitivities

The induction of peroxisome proliferation by xenobiotics is generally determined as an increase in the activities of certain peroxisome-specific enzymes, or as an increase in the numerical or volume density of peroxisomes in the affected organ. Peroxisome proliferation is associated with: increases in number and volume of peroxisomes; an increase in DNA synthesis and liver growth; and liver, Leydig cell, and pancreatic acinar cell tumors. The phenomenon of peroxisome proliferation is not uniform across all species. While rats and mice are particularly sensitive to this phenomenon, guinea pigs, cats, dogs and primates (including man), are predominantly non-responsive.

VI.3. Tumors Associated with C8 in the Rat

C8 has been demonstrated to be a peroxisome proliferator in the rat. C8 exposure in the rat was found to be associated with tumors in the liver, Leydig cell, and pancreatic acinar cell. Peroxisome proliferators, in general, were initially recognized to be associated with hepatocarcinogenesis in rats. However, more recently peroxisome proliferators have been associated with the induction of a triad of tumors in rats: liver, Leydig cell, and pancreatic acinar cell. Hyperplasia of these cell types is typically observed prior to, and along with, the occurrence of neoplasia. Several known peroxisome proliferators (clofibrate, HCFC-123, methylclofenapate, and Wyeth-14,643) are reported to induce this triad of tumors in rats. Hence, this tumor profile appears to be common phenomenon for at least a subset of compounds that are peroxisome proliferators.

VI.3.a. Significance of C8-Induced Rodent Tumor to Human Risk

VI.3.a.1. Liver Tumors

The abundance of data indicates that there is a hepatocarcinogenic hazard of peroxisome proliferators to responsive species (rats and mice) in chronic studies, whereas the carcinogenic hazard to non-responding species, such as humans, is clearly questionable. The epidemiology data, albeit limited, strongly support that the relevance of the hepatocarcinogenic effects of C8 and other peroxisome proliferators for human hazard assessment should be considered negligible.

VI.3.a.2. Leydig Cell Tumors

Leydig cell hyperplasia and adenomas are commonly observed in laboratory rats. The incidence of spontaneous Leydig cell adenomas in Crl:CD®BR rats ranges from approximately 0-12% by 2 years of age, and ranges from approximately 64 -100 % in F344 rats. In contrast, the rate in humans has been reported to be approximately 0.4 per

million (0.00004%). Although a direct comparison is somewhat tenuous, the data suggest a substantial difference in the susceptibility of rodents and humans to Leydig cell tumorigenesis. This is supported by epidemiology data from compounds that clearly produce Leydig cell tumors in rodent studies but are commonly ingested by humans and are not associated with Leydig cell tumorigenesis in humans.

C8 and other peroxisome proliferators do not produce increases in peroxisomes in Leydig cells and are hypothesized to produce these tumors via a different mechanism than the liver tumors. The mechanism of tumorigenesis is not completely understood, and therefore relevance to humans can not be completely ruled out. However, it is known that non-genotoxic compounds (such as C8) produce Leydig cell tumors by altering the endocrine system. Therefore, a threshold for tumorigenesis is expected. If this is the case, use of a margin of safety approach is appropriate for the quantitative dose-response assessment. It is important to consider the slope of the dose-response at the low end of the observed range in determining an acceptable margin of safety.

VI.3.a.3. Pancreatic Acinar Cell Tumors

C8 and other peroxisome proliferators do not produce increases in peroxisomes in the pancreas and are hypothesized to produce these tumors via a different mechanism than the liver tumors. The mechanism of tumorigenesis is not understood, and therefore relevance to humans can not be completely ruled out. However there is a growing weight of evidence that the pancreatic acinar cell tumors are hormonally mediated, therefore they should be treated similarly to peroxisome-proliferator-induced Leydig cell tumors.

VII. SUMMARY

C8 has moderate acute oral toxicity with LD_{50}'s ranging from 178 mg/kg in male guinea pigs to 680mg/kg in adult male rats. An aqueous paste of C8 produced mild to moderate dermal irritation in rabbits and clinical signs of toxicity were observed at doses as low as 1000 mg/kg. Instillation of solid C8 into the rabbit eye produced moderate corneal opacity, iritis, and conjunctivitis. These ocular effects gradually receded. C8 has high acute inhalation toxicity with a 4-hour ALC of 0.8 mg/L in the rat. Subchronic inhalation exposure to C8 produced reversible liver effects at concentrations as low as 8 mg/m^3 (measured as 7.6 mg/m^3). Oral and skin absorption subchronic studies confirmed the hepatotoxicity of C8 in the rat. In chronic feeding studies in rats, C8 produced an increased incidence of tumors in the liver, pancreas, and testis. C8 was found not to be a developmental toxic or mutagenic in several tests for mutagenicity.

The relevance to human health of tumors induced by peroxisome proliferators in rodents has been the focus of several investigators. Regarding the liver, there is a strong association and probable link between peroxisome-proliferator-induced liver growth and the subsequent development of rodent liver tumors. A combination of *in vivo* and *in vitro* studies as well as epidemiology data, has led several investigators to conclude that humans appear to be insensitive or unresponsive to peroxisome-proliferator-induced hepatic effects, and therefore these nongenotoxic agents pose little or no

hepatocarcinogenic hazard to humans. Evidence is also accumulating that the initiating events, which lead to the development of Leydig cell and pancreatic acinar cell tumors are from changes in the liver. These hepatic changes appear to alter the hormonal control of the testis and pancreas. Although these relationships need to be confirmed, it is likely that these extrahepatic tumors pose little or no carcinogenic hazard to humans. Additionally, programs monitoring the health of C8-exposed workers and retrospective cohort studies of workers exposed to C8 provide no evidence of an association between C8 exposure and adverse human health effects.

Of primary concern in humans is the slow clearance of C8 from human blood, the opportunity for exposure in the work place, and the moderate-high acute toxicity, regardless of route of exposure.

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Serum Perfluorooctanoic Acid and Hepatic Enzymes, Lipoproteins, and Cholesterol: A Study of Occupationally Exposed Men

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Perfluorooctanoic acid (PFOA) produces marked hepatic effects, including hepatomegaly, focal hepatocyte necrosis, hypolipidemia, and alteration of hepatic lipid metabolism in a number of animal species. In rodents, PFOA is a peroxisome proliferator, an inducer of members of the cytochrome P450 superfamily and other enzymes involved in xenobiotic metabolism, an uncoupler of oxidative phosphorylation, and may be a cancer promoter. Although PFOA is the major organofluorine compound found in humans, little information is available concerning human responses to PFOA exposure. This study of 115 occupationally exposed workers examined the cross-sectional associations between PFOA and hepatic enzymes, lipoproteins, and cholesterol. The findings indicate that there is no significant clinical hepatic toxicity at the PFOA levels observed in this study. PFOA may modulate the previously described hepatic responses to obesity and xenobiotics. © *1996 Wiley-Liss, Inc.*

KEY WORDS: perfluorooctanoic acid, human, hepatic enzymes, cholesterol, HDL

INTRODUCTION

Perfluorooctanoic acid (PFOA) is a potent synthetic surfactant that is used in a wide variety of industrial processes and products. Organic fluorine has been found in the serum of all human populations studied (Ubel et al., 1980; Taves, 1971; Taves et al., 1976; Guy, 1979, Belisle, 1981). Guy and Taves reported that PFOA was the principal organic fluorine compound in human serum (Taves, 1971; Taves et al., 1976; Guy, 1979). PFOA is found in serum because PFOA has a long biological half-life, allowing accumulation of small doses over time (Ubel et al., 1980). Little is known about the toxic potential of PFOA in humans; however, studies have shown that the liver is an important site of toxicity in animals (Griffith and Long, 1980; Kennedy, 1985; Kennedy et al., 1986; Pastoor et al., 1987; Van Rafelghem et al., 1987; Just et al., 1989).

Animals treated with PFOA rapidly develop hepatomegaly with focal necrosis and show marked hepatic physiologic responses that include hypolipidemia, peroxisome proliferation, induction of xenobiotic metabolic enzymes, increased hepatic tumor incidence, uncoupling of mitochondrial oxidative phosphorylation, and alterations in lipid metabolism (Griffith and Long, 1980; Kennedy, 1985; Kennedy et al., 1986; Pastoor et al., 1987; Van Rafelghem et al., 1987; Just et al., 1989; Takagi et al., 1991, Permadi et al., 1992; Haughom et al., 1992; Sohlenius et al., 1992; Keller et al., 1992; Handler, 1992). Rats treated with PFOA and other peroxisome proliferators (PPs), such as clofibrate, show a 50% reduction of serum cholesterol and changes in the hepatic production and processing of lipoproteins. Haughom et al. (1992) showed that the hypolipidemic response results from downregulation of HMG-CoA reduc-

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TABLE I. Distribution of Exposed Workers by Total Serum Fluorine Category in 3M Chemolite Plant, Cottage Grove, MN

	Total serum fluorine (ppm)					
	<1	1–3	>3–10	>10–15	>15–26	Total
Age[a]	39.9 (10.2)	39.6 (8.5)	36.0 (7.5)	39.3 (11.1)	41.6 (10.5)	39.2
BMI (kg/m^2)[a]	27.6 (5.3)	26.6 (2.6)	26.3 (3.3)	29.4 (3.7)	26.0 (1.4)	26.9
Alcohol use[b]						
<1 oz/day	17 (73.9)	51 (78.5)	9 (56.3)	5 (83.3)	5 (100)	87 (75.6)
1–3 oz/day	2 (8.7)	13 (20.0)	4 (25.0)	1 (16.7)	0 (0)	20 (17.4)
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nonresponse	4 (17.4)	1 (1.5)	3 (18.7)	0 (0)	0 (0)	8 (7.0)
Tobacco use[b]						
Smoker	3 (13.0)	16 (24.6)	6 (37.5)	2 (33.3)	1 (20.0)	85 (73.9)
Nonsmoker	19 (82.7)	49 (75.4)	9 (56.2)	4 (66.7)	4 (80.0)	28 (24.4)
Nonresponse	1 (4.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	2 (1.7)
Total	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)	115

BMI, body mass index.
[a]Values are mean (SD).
[b]Values are n (percent).

tase. In addition, PFOA has been associated with hepatocyte necrosis and increased hepatic enzymes, suggesting that irreversible cell damage occurs (Kennedy, 1985; Just et al., 1989). Hepatomegaly and alterations in lipid metabolism appear to be rapidly reversible; however, other hepatic changes are not rapidly reversed (Perkins, 1992; Sohlenius et al., 1992).

Based on findings from the studies of rodents and in vitro experiments, some investigators have suggested that PFOA is likely to present a health risk to humans (Just et al., 1989; Takagi et al., 1991). If the observations in rodent species are relevant to humans exposed to PFOA, it is reasonable to hypothesize that changes in human hepatic enzymes and lipid metabolism are similar to those observed in rodents. Limited data are available to assess the hepatic responses to PFOA in humans. Ubel and coworkers (1980) and Griffith and Long (1980) reported that PFOA-exposed workers showed no clinical evidence of adverse hepatic effects. Furthermore, a retrospective cohort mortality study of exposed workers found no excess mortality from liver cancer or liver disease (Gilliland and Mandel, 1993). To assess whether the changes in cholesterol, lipoproteins, and hepatic enzymes observed in rodents treated with PFOA occur in humans, we studied 115 occupationally exposed employees at a plant that produces PFOA. Production workers with the highest PFOA exposures had serum PFOA levels similar to those in rodents that developed hepatomegaly when treated orally with low doses of PFOA (Ubel et al., 1980). We examined the cross-sectional association between serum PFOA, a validated surrogate measure of total serum fluorine, and cholesterol, lipoproteins, and hepatic enzymes in this group of occupationally exposed men.

TABLE II. Distribution of Age, Alcohol, and Tobacco Use in Participants by Body Mass Index in Study of Workers Exposed to PFOA

	BMI mg/kg^2		
	<25	25–30	>30
Total	41 (100%)	57 (100%)	17 (100%)
Tobacco use			
Smoker	11 (26.8%)	15 (26.3%)	2 (11.8%)
Nonsmoker	29 (70.7%)	41 (71.9%)	15 (88.2%)
Nonresponse	1 (2.5%)	1 (1.8%)	0 (0%)
Alcohol use			
<1 oz/day	31 (75.6%)	43 (75.4%)	13 (76.4%)
1–3 oz/day	6 (14.6%)	11 (19.3%)	3 (17.7%)
Nonresponse	4 (9.8%)	3 (5.3%)	1 (5.9%)
Age			
		2	
<40 years	31 (75.6%)	8 (49.1%)	6 (35.3%)[a]
≥40 years	10 (24.4%)	29 (50.9%)	11 (64.7%)
Total serum flourine			
Mean ppm (SD)	2.8 (3.7)	4.0 (5.5)	2.1 (3.5)

[a]$p = .005$.
BMI, body mass index.

MATERIALS AND METHODS

Subject Selection

Participants were recruited from current employees at a PFOA production plant that has operated since 1947. The

TABLE III. Serum Cholesterol, Low Density Lipoprotein, and High Density Lipoprotein by Total Serum Fluoride in Study of Workers Exposed to PFOA

Total fluoride	N	Mean	SD	Median	Range	Test[a]
Cholesterol (mg/dl)						
<1 ppm	23	201	34.7	203	132–268	F = .066
≥1–3	65	211	40.0	212	130–349	p = .62
>3–10	16	206	37.7	198	150–277	
>10–15	6	226	40.0	216	183–298	
>15–26	5	214	27.0	204	184–244	
Total	115	210	38.1	210	130–349	
LDL (mg/dl)						
<1 ppm	23	132	32.4	137	70–196	F = 0.31
≥1–3	65	136	34.5	131	70–264	p = .87
>3–10	16	134	34.5	133.5	83–217	
>10–15	6	124	44.0	139	36–156	
>15–26	5	143	20.8	144	117–171	
Total	115	135	33.8	134	36–264	
HDL (mg/dl)						
<1 ppm	23	45.9	11.7	47	19–67	F = 0.66
≥1–3	65	46.1	10.0	44	30–79	p = .66
>3–10	16	41.8	10.2	40	29–68	
>10–15	6	46.5	6.8	44	40–59	
>15–26	5	45.6	10.2	49	29–54	
Total	115	45.4	10.2	43	19–79	

[a]Anova.
LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE IV. Pearson Correlation Coefficients Between Total Serum Fluoride, Age, Body Mass Index, Daily Alcohol Use, Daily Tobacco Consumption, and Lipoproteins

	Total fluoride (ppm)	Age (years)	BMI (kg/m^2)	Alcohol (oz/day)	Tobacco (cigs/day)
CHOLESTEROL	.07	.25 p = .008	.19 p = .05	.09	.35 p = .0001
LDL	.02	.13	.06	−.008	.28 p = .00
HDL	−.01	.03	−.13	.18 p = .06	−.09

LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

TABLE V. Serum Cholesterol by Body Mass Index, Age, Smoking, and Drinking Status: 3M Chemolite Plant, Cottage Grove, Minnesota

		Cholesterol (mg/dl)				
	N (%)	Mean	SD	Median	Range	Test[a]
BMI						
<25	41 (35.7)	195	40.1	186	130–277	F = 5.10
25–30	57 (49.6)	219	36.2	220	146–349	p = .008
>30	17 (14.8)	214	29.3	216	163–268	
Age						
≤30	21 (18.3)	196	37.8	201	130–254	F = 1.60
>30–40	48 (41.7)	2.9	43.8	204	132–349	p = .19
>40–50	27 (23.5)	216	30.2	216	163–263	
>50–60	19 (16.5)	219	29.7	224	164–268	
Alcohol						
<1 oz/d	87 (81.3)	209	38.6	204	135–349	F = .63
1–3 oz/d	20 (18.7)	216	33.5	218	130–277	p = .43
Missing	8	207	45.5	213	132–261	
Tobacco						
Smoker	28 (24.8)	233	41.6	238	167–349	F = 15.63
Nonsmoker	85 (75.2)	203	32.9	203	130–268	p = .0001
Missing	2	198	89.1	198	135–261	
Total	115					

[a]Anova.
BMI, body mass index.

plant produces a number of specialty chemicals in addition to PFOA. Details about the plant have been described previously (Gilliland and Mandel, 1993). All workers employed in PFOA production during the period 1985–1989 were invited to participate in the study. Workers with jobs involving direct contact with PFOA during the 1985–1989 period were considered highly exposed. This group included maintenance and engineering supervisors, as well as production workers. Forty-eight (96%) of 50 exposed workers agreed to participate in the study. In addition, a sample of workers employed in jobs with no apparent PFOA exposure was asked to participate. Those without direct contact with PFOA for at least 5 years were considered to have low exposure. A randomly selected low-exposure group of workers was frequency matched in 5-year age groups to the high-exposure workers. Sixty-five employees from jobs thought to involve no PFOA exposure volunteered for the study. The total number of the presumed unexposed employees invited to participate was not recorded; however, few individuals in this group declined to participate. We estimate that more than 80% of those invited agreed to participate in the study.

Total serum fluorine was used as a surrogate variable for PFOA exposure. We assayed total serum fluorine rather than measuring PFOA directly because the assay was less expensive and technically easier to perform on the large number of samples collected in this study. Furthermore, the use of total serum fluorine has been validated as a surrogate marker for PFOA in past biological monitoring in the plant and other plants using PFOA (Ubel et al., 1980). Approximately 90% of total serum fluorine in workers was reported

TABLE VI. Serum Low Density Lipoprotein by Body Mass Index, Age, Smoking, and Drinking Status: 3M Chemolite Plant, Cottage Grove, Minnesota

		LDL(mg/dl)				
	N (%)	Mean	SD	Median	Range	Test[a]
BMI						
<25	41 (35.7)	130	22.8	133	70–217	F = .65
25–30	57 (49.6)	138	34.2	135	36–264	p = .52
>30	17 (14.8)	136	33.0	137	71–196	
Age						
≤30	21 (18.3)	130	29.6	131	75–177	F = .37
>30–40	48 (41.7)	136	36.2	135	70–264	p = .77
>40–50	27 (23.5)	133	34.5	135	36–193	
>50–60	19 (16.5)	140	32.3	137	20–196	
Alcohol						
<1 oz/d	87 (81.3)	135	34.5	133	36–264	F = .01
1–33 oz/d	20 (18.7)	135	31.4	137	78–217	p = .93
Missing	8	134	35.6	141	70–174	
Tobacco						
Smoker	28 (24.8)	152	35.6	146	99–264	F = 9.42
Nonsmoker	85 (75.2)	130	31.3	133	78–217	p = .003
Missing	2	115	55.9	115	70–174	
Total	115					

[a]Anova.
BMI, body mass index; LDL, low density lipoprotein.

TABLE VII. Serum High Density Lipoprotein by Body Mass Index, Age, Smoking, and Drinking Status

		HDL (mg/dl)				
	N (%)	Mean	SD	Median	Range	Test[a]
BMI						
<25	41 (35.7)	46.0	10.7	43	19–68	F = .38
25–30	57 (49.6)	45.6	10.5	44	22–79	p = .69
>30	17 (14.8)	43.6	7.7	43	32–55	
Age						
≤30	21 (18.3)	43.5	14.3	40	19–79	F = .72
>30–40	48 (41.7)	46.7	9.9	46	22–65	p = .55
>40–50	27 (23.5)	46.0	8.3	45	29–61	
>50–60	19 (16.5)	46.6	7.9	43	32–67	
Alcohol						
<1 oz/d	87 (81.3)	44.3	9.2	43	19–65	F = 3.88
1–3 oz/d	20 (18.7)	49.3	13.5	45	29–79	p = .05
Missing	8	48.3	9.3	53	32–55	
Tobacco						
Smoker	28 (24.8)	44.3	8.9	43	29–68	F = .35
Nonsmoker	85 (75.2)	45.6	10.6	43	19–79	p = .56
Missing	2	54.5	21.2	55	53–56	
Total	115					

[a]Anova.
BMI, body mass index; HDL, high density lipoprotein.

to be in the form of PFOA (Venkateswarlu, 1982). Because the vast majority of total serum fluorine in plant employees is in the form of PFOA, total serum fluorine closely reflects serum PFOA in production workers, and its use is unlikely to introduce substantial error into the study.

We expected the group of workers who were selected for the unexposed group based on job history to have total serum fluorine levels similar to the general population. However, we found that this group of workers was not unexposed, having levels 20–50 times higher than levels reported for the general population. We concluded that job history was not an accurate metric for exposure. Because job history performed poorly for exposure assessment, we used measured total serum fluorine to classify individuals in the analyses.

Data Collection

Participants completed a medical history questionnaire, were measured for height and weight, and donated a blood sample by venipuncture for assays of total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The blood sample for total serum fluorine (TSF) was collected in a fluorine-free 15-ml Vacutainer. Divided aliquots of serum collected for total fluorine assay were frozen at −70°C. After all total fluorine samples had been received, batches of 15 samples were assayed on successive work days. Total serum fluorine, reported as a mean value, was determined using sodium biphenyl extraction and atomic absorption spectroscopy (Venkateswarlu, 1982). Each sample was assayed twice. Each batch included high- and low-quality control samples.

Analysis

Stratified analysis, Anova, Pearson correlation coefficients, and linear multivariate regression were used to evaluate associations between PFOA and the biochemical endpoints. For stratified analyses, Anova procedures were used to assess differences in mean values. Total serum fluorine was divided a priori into five categories—<1 ppm, 1–3 ppm, >3–10 ppm, >10–15 ppm, and >15 ppm—based on the distribution of previous monitoring data. Age, body mass index (BMI), alcohol use, and tobacco use were included in regression models as potential confounders. Number of cigarettes smoked per day was used as a continuous

TABLE VIII. Linear Multivariate Regression Model of Factors Predicting the High Density Lipoprotein in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	65.00	10.07	.0001
Total fluorine	−1.61	.77	.04
Alcohol[a]			
Low (<1 oz/day)	−9.92	3.51	.006
Nonresponsive (NR)	−6.77	5.73	.24
Low × total fluorine[b]	1.62	.80	.04
NR × total fluorine[b]	2.05	1.63	.21

R^2 = .17.
[a]Reference category is drinkers who consumed 1–3 oz ethanol/day.
[b]Interaction terms between total fluorine and alcohol category.
Adjusted for age, body mass index, smoking, and testosterone.

TABLE IX. Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transminase, and Gamma Glutamyl Transferase by Total Serum Fluorine in Study of Workers Exposed to PFOA

Total fluorine	N	Mean	SD	Median	Range	Test[a]
SGOT (IU/dl)						
<1 ppm	23	22.5	4.1	22	13–29	F = 0.41
≥1–3	65	24.1	8.6	23	10–74	p = .80
>3–10	16	25.8	14.5	22.5	17–77	
>10–15	6	25.7	11.3	22.5	17–47	
>15–26	5	22.2	5.1	22	14–27	
SGPT (IU/dl)						
<1	23	47.7	10.7	46	30–69	F = 1.19
≥1–3	65	51.3	30.2	45	4–263	p = .32
>3–10	16	53.0	14.0	50.5	29–40	
>10–15	6	73.2	53.2	52.5	38–177	
>15–26	5	44.6	8.6	42	34–54	
GGT (IU/dl)						
<1 ppm	23	37.2	29.4	27	6–117	F = 0.39
≥1–3	65	32.4	26.7	25	5–174	p = .81
>3–10	16	35.4	35.4	26	10–158	
>10–15	6	38.3	16.7	36.5	19–60	
>15–26	5	22.2	11.5	20	11–37	
Total	115	33.7	27.6	26	5–174	

[a]Anova.
SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GGT, gamma glutamyl transferase.

TABLE X. Pearson Correlation Coefficients Between Total Serum Fluorine, Age, Body Mass Index, Daily Alcohol Use, Daily Tobacco Consumption, and Hepatic Parameters in Study of Workers Exposed to PFOA

	Total fluorine (ppm)	Age (years)	BMI (kg/m²)	Alcohol (oz/day)	Tobacco (cigs/day)
SGOT	.01	−.10	.09	.12	−.11
SGPT	.01	.01	.20	.03	−.11
			p = .02		
GGT	−.04	.12	.27	.15	.03
			p = .004		

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GGT, gamma glutamyl transferase; BMI, body mass index.

TABLE XI. Serum Glutamic Oxaloacetic Transaminase by Body Mass Index, Age, Smoking, and Drinking Status in Study of Workers Exposed to PFOA

	SGOT (IU/dl)					
	N (%)	Mean	SD	Median	Range	Test[a]
BMI						
<25	41 (35.7)	24	12.4	22	13–77	F = .92
25–30	57 (49.6)	23	5.8	23	10–42	p = .40
>30	17 (14.8)	27	8.1	26	17–47	
Age						
≤30	21 (18.3)	25	12.7	23	17–77	F = .78
>30–40	48 (41.7)	24	9.1	23	10–74	p = .51
>40–50	27 (23.5)	22	5.4	23	13–40	
>50–60	19 (16.5)	26	7.8	23	14–47	
Alcohol						
<1 oz/d	87 (81.3)	26	13.5	22	16–77	F = .61
1–3 oz/d	20 (18.7)	24	8.0	23	10–74	p = .44
Missing	8	23	4.3	21	19–31	
Tobacco						
Smoker	28 (24.8)	24	8.4	23	13–77	F = .02
Nonsmoker	85 (75.2)	24	11.0	22	10–42	p = .89
Missing	2	20	3.5	20	17–47	
Total	115					

[a]Anova.
SGOT, serum glutamic oxaloacetic transaminase; BMI, body mass index.

variable if model fit was improved compared with the model using categorical variables. BMI was categorized into three categories, <25 kg/m^2, 25–30 kg/m^2, and >30 kg/m^2. Alcohol use was divided into three categories: <1 drink per day, 1–3 drinks per day, and no response to the questionnaire item, and was entered into the models as a set of indicator variables. Significant nonlinear dose-response relationships were evaluated by comparing model fit using residual analysis and by comparing parameter estimates using indicator variables and continuous variables. Interactions between total serum fluorine and the covariates were evaluated based on biologic plausibility. Interaction terms were included in the final model if the parameter estimate had a p value ≤0.05. The two nonrespondents to the smok-

TABLE XII. Serum Glutamic Pyruvic Transaminase by Body Mass Index, Age, Smoking, and Drinking Status: 3M Chemolite Plant, Cottage Grove, Minnesota

		SGPT (IU/dl)				
	N (%)	Mean	SD	Median	Range	Test
BMI						
<25	41 (35.7)	49	35.4	41	29–263	F = 2.1
25–30	57 (49.6)	50	14.2	49	4–95	p = .12
>30	17 (14.8)	64	32.8	55	38–177	
Age						
≤30	21 (18.3)	49	11.5	45	31–80	F = .61
>30–40	48 (41.7)	53	33.6	47	29–263	p = .61
>40–50	27 (23.5)	47	15.2	46	4–99	
>50–60	19 (16.5)	57	32.0	50	34–177	
Alcohol						
<1 oz/d	87 (81.3)	53	2935	47	29–263	F = .68
1–3 oz/d	20 (18.7)	47	16.9	46	4–99	p = .41
Missing	8	51	10.9	52	35–67	
Tobacco						
Smoker	28 (24.8)	48	15.2	47	4–90	F = .76
Nonsmoker	85 (75.2)	53	29.6	48	30–263	p = .39
Missing	2	49	25.5	49	31–67	
Total	115					

[a]Anova.
BMI, body mass index; SGPT, serum glutamic pyruvate transaminase.

TABLE XIII. Gamma Glutamyl Transferase by Body Mass Index, Age, Smoking, and Drinking Status in Study of Workers Exposed to PFOA

		GGT (IU/dl)				
	N (%)	Mean	SD	Median	Range	Test[a]
BMI						
<25	41 (35.7)	28	31.1	17	5–174	F = 3.54
25–30	57 (49.6)	34	23.1	19	8–158	p = .03
>30	17 (14.8)	48	28.6	44	19–117	
Age						
≤30	21 (18.3)	32	23.4	25	11–111	F = 1.58
>30–40	48 (41.7)	31	32.7	22	5–174	p = .36
>40–50	27 (23.5)	33	17.2	29	8–72	
>50–60	19 (16.5)	44	29.3	35	11–117	
Alcohol						
<1 oz/d	87 (81.3)	40	25.5	35	8–89	F = 1.64
1–3 oz/d	20 (18.7)	32	25.3	26	6–174	p = .36
Missing	8	41	50.4	23	12–158	
Tobacco						
Smoker	28 (24.8)	36	21.3	33	5–89	F = .55
Nonsmoker	85 (75.2)	32	26.3	25	6–174	p = .46
Missing	2	85	103.2	85	12–158	
Total	115					

[a]Anova.
BMI, body mass index; GGT, gamma glutamyl transferase.

ing questions were not included in the analysis. All analyses were conducted using the statistical computing package SAS (Statistical Analysis Systems, 1992).

RESULTS

Participant characteristics are shown in Tables I and II. Total serum fluorine values for the 115 participants varied between 0 and 26 ppm, with a mean of 3.3 ppm. Twenty-three (20.0%) participants had serum values <1 ppm, and 11 (9.6%) had values >10 ppm (Table I). The distributions for age, BMI, alcohol use, and tobacco use did not differ significantly among total serum fluorine categories. Mean total serum fluorine, tobacco use, and alcohol use did not differ significantly between obese and non-obese workers. Obese (BMI > 30) participants were significantly older than non-obese participants (Table II).

In univariate analyses, the marked hypolipidemic effect of PFOA observed in rodents was not apparent in exposed workers (Table III). As shown in Table IV, total serum fluorine was not significantly correlated with cholesterol, LDL, or HDL; however, several expected correlations were present. Alcohol consumption was associated with higher HDL levels. Age and body mass index (BMI) were significantly correlated with cholesterol. The number of cigarettes smoked per day was significantly correlated with cholesterol and LDL. A similar pattern of associations was seen in the stratified analyses for these variables (Tables V–VII).

We found that PFOA was associated with HDL levels in moderate drinkers. After adjusting for alcohol use, age, BMI, cigarette use, and testosterone levels, moderate alcohol use was associated with an increase in HDL (9.9 mg/dl) compared with light drinkers or abstainers (Table VIII). As total serum fluorine increased, the effect of moderate alcohol use on HDL was blunted; a 10-ppm rise in total serum fluorine reversed the effect of moderate alcohol on HDL. In light drinkers, little change in HDL was observed as total fluorine increased. After adjusting for alcohol use, age, BMI, and cigarette use, total serum fluorine was not significantly associated with cholesterol, or LDL (not shown).

SGOT, SGPT, and GGT did not significantly differ among the five categories of total serum fluorine (Table IX). As expected, SGPT and GGT were significantly correlated with BMI, but were not significantly correlated with total serum fluorine, age, alcohol consumption, or cigarette consumption (Table XI). Stratified analyses indicated the same pattern, except GGT was also associated with BMI (Tables IX–XIII).

After adjusting for age, cigarette use, alcohol use, and

TABLE XIV. Linear Multivariate Regression Model of Factors Predicting Serum Glutamic Oxaloacetic Transaminase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	26.71	7.1	.0003
Total fluorine (ppm)	−3.23	1.31	.02
BMI (kg/m^2)	−.0004	.23	.99
BMI × total fluorine[a]	.12	.05	.015

R^2 = .17.
[a]Interaction term between total serum fluorine and BMI.
Adjusted for age, alcohol use, and smoking.
BMI, body mass index.

TABLE XV. Linear Multivariate Regression Model of Factors Predicting Serum Glutamic Pyruvic Transaminase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	58.13	24.6	.02
Total fluorine (ppm)	−15.80	4.58	.0008
BMI (kg/m^2)	.30	.82	.72
BMI × total fluorine[a]	.62	.17	.0004

R^2 = .21.
[a]Interaction term between total serum fluorine and BMI.
Adjusted for age, alcohol use, and smoking.
BMI, body mass index.

TABLE XVI. Change in Serum Glutamic Oxaloacetic Transaminase and Serum Glutamic Pyruvic Transaminase Associated With a 10 ppm Change in Total Serum Fluorine in Study of Workers Exposed to PFOA

BMI (kg/m^2)	25	30	35
SGOT	−2.4	3.7	9.7
SGPT	−3.0	28.0	59.0

BMI, body mass index; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

BMI, total serum fluorine was associated with changes in SGOT, SGPT, and GGT through interactions with known determinants of hepatic enzymes. SGOT and SGPT showed a different association in obese and non-obese workers (Tables XIV and XV). A 10 ppm difference in total serum fluorine resulted in a slight decrease in SGOT and SGPT for non-obese (BMI = 25 kg/m^2) workers (Table XVI). In obese (BMI = 35 kg/m^2) workers, an increase in SGOT and SGPT was associated with a 10 ppm difference. In the regression model for GGT (Table XVII), moderate alcohol consumption was positively associated with GGT. In moderate drinkers, GGT decreased as total fluorine increased; the decrease was less in light drinkers.

TABLE XVII. Linear Multivariate Regression Model of Factors Predicting Gamma Glutamyl Transferase in Study of Workers Exposed to PFOA

Variable	β	SE(β)	p value
Intercept	−12.59	22.62	.58
Total fluorine (ppm)	−1.93	2.11	.36
Alcohol[a]			
Low (<1 oz/day)	−12.37	9.50	.20
Nonresponse (NR)	−28.13	15.46	.07
Low × fluorine[b]	1.59	2.18	.47
NR × fluorine[b]	13.90	4.48	.003

R^2 = .18.
[a]Reference category is drinkers who consumed 1–3 oz ethanol/day.
[b]Interaction terms between total fluorine and alcohol category.
Adjusted for age, body mass index, and smoking.

All hepatic enzyme assays were in a clinically acceptable range, and no workers reported hepatic disease diagnoses or signs, or symptoms consistent with hepatic disorders. No clinical cases of liver dysfunction associated with PFOA exposure have been found by the medical surveillance program at the plant.

DISCUSSION

PFOA is an alleged cancer promoter in rats (Reddy et al., 1980). In biphasic liver carcinogenesis protocols (initiation and promotion) and triphasic protocols (initiation, selection, and promotion), PFOA produced increased numbers of malignant hepatocellular carcinomas (Abdellatif et al., 1990; Nilsson et al., 1991). Takagi et al. (1991) have suggested that because the intensity of hepatic response may be an early marker for liver carcinogenic potential, PFOA has a high potential for liver carcinogenesis. The hypolipidemia observed in PFOA-treated rodents was not observed in PFOA-exposed workers. At the levels of exposure in this study, PFOA is not associated with a marked hepatic response and is not likely to have a significant carcinogenic potential in humans. Obese workers may be a susceptible population for subclinical hepatic changes.

In rodents, PFOA alters endobiotic and xenobiotic hepatic metabolic enzyme profiles (Pastoor et al., 1987). Few studies of the human response to PFOA exposure have been published. In a study at the same plant, Ubel et al. (1980) reported no association between PFOA and hepatic enzymes. However, their analysis did not consider the joint effects of obesity or alcohol with PFOA exposure. In the present study, changes in SGOT and SGPT were associated with PFOA through an interaction with adiposity. In obese

participants only, SGOT and SGPT increased with increasing PFOA. The hypothesis that PFOA may modulate the hepatic effects of obesity is consistent with these changes in enzyme profile. This hypothesis has biologic plausibility because obesity has been associated with elevation of transaminases through fatty infiltration (Ludwig et al., 1980; Hodgson et al., 1989). PFOA may directly or indirectly potentate this effect in susceptible individuals. PFOA alters hepatic lipid metabolism and may block the metabolism of accumulated fatty acids, resulting in an exacerbation of the pathologic process (Haughom et al., 1992).

PFOA may also modulate the effect of alcohol on hepatic metabolism. PFOA is associated with changes in the effect of alcohol consumption on HDL levels, essentially blocking the rise in HDL associated with alcohol consumption. GGT was inversely associated with PFOA in drinkers. Perfluorooctanoic acid may decrease serum GGT by altering cell membrane permeability, by reducing the alcohol-mediated induction of GGT, or by changing alcohol oxidation pathways and reducing the production of such toxic intermediates as acetaldehyde (Bates, 1981; Schuckit and Griffiths, 1982; Orrego et al., 1985; Schuckit and Irwin, 1988). These findings support the hypothesis that PFOA modulates the effects of endogenous and exogenous determinants of hepatic metabolism.

Interpretation of these findings is limited by a number of factors. Only active workers in PFOA production were included in this study. It is unlikely that workers who had significant exposure during the previous 5 years would have been lost to this study because of transfer out of the PFOA production division. Transfer as a result of subclinical changes in such biochemical parameters as SGOT is unlikely. Because of the low turnover rate in plant employees (3% per year) and the inclusion of most current employees with appropriate job histories, selection bias is not a likely explanation for the findings in this study. Given the high participation (>80%), nonresponse bias is likely to be small. Information on smoking and alcohol consumption was collected and used in the analyses; however, measurement error for these variables could allow residual confounding. Because smoking and alcohol consumption are not strong determinants for the endpoints in this study, the magnitude of any residual confounding is likely to be small. The duration of exposure may be an important determinant of PFOA level and effect; however, information on the duration of employment in exposed jobs was not available because plant records did not contain sufficient information to reconstruct exposures. Furthermore, the use of job history resulted in marked misclassification of exposure status, indicating that the use of job duration would be of limited value in determining duration of exposure.

Many of the participants were employed in the production of compounds other than PFOA; however, none of these processes involve substantial exposure to known hepatotoxins. Because PFOA has a long biological half-life in humans, is absorbed easily, and is hepatotoxic in rodents, PFOA production workers have been under medical surveillance for more than 20 years. No adverse clinical outcomes related to PFOA exposure have been observed in these employees.

In summary, PFOA was not associated with the marked hepatic changes in humans that have been observed in rodents. This finding is consistent with the results of a retrospective mortality study that found no increased mortality from liver disease (Gilliland and Mandel, 1993) and with the results from an earlier morbidity study that found no adverse hepatic effects (Ubel et al., 1980). PFOA may modulate the effect of alcohol use and obesity on hepatic lipid and xenobiotic metabolism. Continued epidemiologic surveillance is appropriate in workers exposed to PFOA.

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1166 ACUTE DOSING OF PEROXISOME PROLIFERATORS INCREASES EXPRESSION OF HEPATIC p34^{cdc2} IN RATS. X Mayol, J G Rabish. Paracelsian, Inc., Ithaca, NY.

Research into the regulation of the cell cycle has shown that the progression of the cell from G_0 to G_1 and through M phase is controlled by a group of enzymes collectively termed cyclin-dependent kinases (CDK). The peroxisome proliferators Wyeth-14643 (WY), clofibrate (CL) and diethylhexylphthalate (DEHP) were examined for their ability to induce changes in the intracellular protein expression of an hepatic CDK and PCNA, an established biomarker for cell proliferation. Young, male rats were given i.p. injections of 50 mg WY/kg for 1, 2 or 3 days or 162 mg CL/kg for 1 day. DEHP was administered as 500 mg DEHP/kg for 1 day or twice daily doses of 500 mg DEHP/kg for 5 days. ELISA and immunoblotting methodology using anti-CDK and anti-PCNA antibodies were used to describe the time course of hepatic CDK and PCNA expression. Compared to controls, WY increased hepatic CDK content 3.6-, 5.0- and 35-fold over the three dosing days. Hepatocyte PCNA content was increased 5.7-, 33- and 89-fold relative to controls over the same three days. By postdosing day 1, CL and DEHP had increased ($p<0.05$) the expression of rat hepatic CDK 2- and 1.9-fold, respectively, compared to controls. Hepatic PCNA expression was increased ($p<0.05$) 1.5-fold over the controls by both CL and DEHP. Five day dosing of DEHP increased expression of CDK and PCNA 43- and 73-fold, respectively, relative to the controls. The increase in expression of CDK common to all three peroxisome proliferators tested in this study indicates that the proliferative stimulus of the these compounds may be quantified as an increase in hepatic CDK expression.

1167 EFFECT OF METHYLCLOFENAPATE (MCP) ON PEROXISOME PROLIFERATION, CELL REPLICATION AND TRANSFORMING GROWTH FACTOR ß1 (TGF-ß1) GENE EXPRESSION IN RAT LIVER. B Q Lake, P C Rumsby, M E Cunningham, M J Davies and R J Price. BIBRA Toxicology International, Carshalton, Surrey, England.

A wide variety of chemicals including MCP have been shown to produce hepatomegaly, peroxisome proliferation and liver tumours in the rat. Male Sprague Dawley rats were fed diets containing 0(control), 0.00025, 0.0005, 0.001, 0.005, 0.015, 0.025, 0.05 and 0.075% MCP for 1, 4 and 13 wk. MCP produced dose-related increases in relative liver weight and activities of peroxisomal and microsomal fatty acid oxidising enzymes at all time points. Significant increases in relative liver weight and enzyme activities were observed at MCP doses as low as 0.001 and 0.00025%, respectively. Replicative DNA synthesis was studied by implanting 7-day osmotic pumps containing 5-bromo-2'-deoxyuridine during study wk 0-1, 3-4 and 12-13. Hepatocyte Labelling Index (LI) values were significantly increased by treatment with 0.001-0.075% MCP for 1 wk. After 4 and 13 wk treatment LI values were still significantly increased in rats given 0.015-0.075% MCP. TGF-ß1 mRNA expression was increased at all time points, being 1.5-1.7 fold control levels in rats given 0.005-0.075% MCP for 4 and 13 wk. These results demonstrate that while low doses of MCP produce sustained hepatomegaly and peroxisome proliferation, higher doses are required to produce a sustained stimulation of replicative DNA synthesis. MCP also increases mRNA levels of TGF-ß1 which is known to inhibit hepatocyte replication and trigger apoptosis. The balance between effects on cell replication and cell death may be an important factor in the mechanism of peroxisome proliferator-induced hepatocarcinogenesis. (Supported by the U.K. Ministry of Agriculture, Fisheries and Food)

1168 DICHLOROACETIC ACID (DCA) AND TRICHLOROACETIC ACID (TCA) PROMOTION OF METHYLNITROSUREA (MNU)-INITIATED TUMORS AND EFFECT ON CELL PROLIFERATION IN LIVER OF B6C3F1 MICE. J B Phelps and M A Pereira. EHRT, Inc., Lexington, KY.

DCA and TCA are formed during chlorination of drinking water and are metabolites of trichloroethylene and tetrachloroethylene. They are hepatocarcinogens in mice and proposed tumor promoters. Female B6C3F1 mice were initiated on day 15 of age with 25 mg/kg MNU. DCA at 0.26, 0.86, or 2.6 and TCA at 0.32, 1.06, or 3.2 g/liter were administered in the drinking water starting at 4 wks. of age. Mice were sacrificed at 5, 12, 33 and 221 days later. Five days prior to sacrifice, minipumps containing 30 mg/ml bromodeoxyuridine (BrDU) were implanted subcutaneously. DCA was a more potent promoter of MNU-initiated liver foci than TCA, while both were of similar potency as promoters of tumors. TCA to a greater extent than DCA, increased the BrDU labeling index after 5 days of exposure, while neither agent was active at later times. In conclusion, DCA and TCA were demonstrated to be tumor promoters and to enhance cell proliferation for which sustained enhancement in precanceous cells could be the mechanism for hepatocarcinogenicity.

1169 MECHANISMS OF EXTRAHEPATIC TUMOR INDUCTION BY PEROXISOME PROLIFERATORS IN Crl:CD®BR (CD) RATS. J C Cook, M E Hurtt, S R Frame, and L B Biegel. DuPont Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE

Wyeth-14,643 (WY) and ammonium perfluorooctanoate (C8) belong to a diverse class of compounds which have been shown to produce hepatic peroxisome proliferation and hepatocellular carcinoma in rats. Based on a review of bioassay data, a relationship appears to exist between peroxisome proliferating compounds and Leydig cell adenoma formation. To further investigate this relationship, a 2-year feeding study in male CD rats was conducted using 50 ppm WY and 300 ppm C8. In addition to the ad libitum control, a second control was pair-fed to the C8 group. Peroxisome proliferation (ß-oxidation activity) and cell proliferation (BrdU, 6-day osmotic pumps) were measured in the liver and testis. Serum testosterone, estradiol, prolactin, LH, and FSH levels were also measured. Interim sacrifices were performed at 3 month intervals as well as at 1 month. Increased relative liver weights and hepatic ß-oxidation activity were observed at all timepoints in both the WY- and C8-treated rats. In contrast, hepatic cell proliferation was significantly increased only in the WY-treated rats. Neither WY nor C8 significantly altered the rate of Leydig cell ß-oxidation or Leydig cell proliferation when compared to the control groups. Moreover, the rate of ß-oxidation in Leydig cells was approximately 20-times less than the rate of hepatic ß-oxidation, regardless of treatment. The most consistent endocrine changes induced by C8 and WY were an increase in estradiol and a decrease in prolactin levels. Histopathological evaluation revealed compound-related increases in liver, Leydig cell, and pancreatic acinar cell tumors in both WY- and C8-treated rats. Based on our data, the Leydig cell tumors appear to be due to the combination of elevated estradiol levels and reduced prolactin levels. Preliminary data suggest that the pancreatic acinar cell tumors are related to an increase in serum cholecystokinin (CCK) levels.

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Mortality Among Employees of a Perfluorooctanoic Acid Production Plant

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Perfluorooctanoic acid (PFOA) has been found at low levels (10 to 100 parts per billion) in sera of the general population and at higher levels in occupationally exposed workers. Although PFOA has been reported to be a promoter of rodent hepatocarcinogenesis and to alter reproductive hormones in humans and rodents, there is little information on human health effects associated with PFOA exposure. The present study examined the relationship between PFOA and mortality using a retrospective cohort mortality design. The cohort consisted of 2788 male and 749 female workers employed between 1947 and 1983 at a plant that produced PFOA. The all-causes standardized mortality ratio was .75 (95% confidence interval [CI], .56 to .99) for women and .77 (95% CI, .69 to .86) for men. Among men the cardiovascular standardized mortality rate was .68 (95% CI, .58 to .80) and the all-gastrointestinal diseases was .57 (95% CI, .29 to .99). There was no significantly increased cause-specific standardized mortality ratio for either men or women. Ten years of employment in exposed jobs was associated with a 3.3-fold increase (95% CI, 1.02 to 10.6) in prostate cancer mortality compared to no employment in PFOA production. There were only six prostate cancer deaths overall and four among the exposed workers; thus, the results must be interpreted cautiously. If prostate cancer mortality is related to PFOA, PFOA may increase prostate cancer mortality by altering reproductive hormones in male workers.

Perfluorooctanoic acid (PFOA) and its salt, ammonium perfluorooctanoate, are perfluorinated surfactants. Because of their unique surface active properties they are used in a large number of industrial applications and consumer products including plasticizers, lubricants, wetting agents, and emulsifiers.[1-3] Despite their widespread use, little is known about potential adverse health effects.

PFOA induced marked hepatomegaly and peroxisome proliferation in rodent livers.[3-8] The chemically diverse group of xenobiotics that induce peroxisomes is of concern because of its association with nongenotoxic hepatocarcinogenesis.[3-10] PFOA did not produce an increased number of hepatocellular carcinomas in a 2-year rat feeding study.[8] However, biphasic (initiation and promotion) and triphasic (initiation, selection, and promotion) hepatic carcinogenesis studies in rodents have shown significantly increased numbers of carcinomas in the PFOA-treated rats.[11,12] It has been suggested that the marked rodent hepatomegaly produced by PFOA is a marker for carcinogenic potential.[13] The observations of increased Leydig cell tumors in a 2-year rat PFOA feeding study and of disruption of the hypothalamic-pituitary-gonad axis in PFOA-treated rats[8] are consistent with the hypothesis that PFOA-associated tumors are mediated by a hormonal nongenotoxic mechanism.

PFOA has a long half-life in humans. A study of occupationally exposed workers showed that the half-life in men is greater than 1.5 years.[14] Hence, accumulation of PFOA may occur from small, frequent PFOA doses. PFOA in the serum of the gen-

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eral populations of industrialized countries[15–19] is likely to be the result of an accumulation of small PFOA doses.

No health problems related to PFOA exposure were observed in a cross-sectional study among workers employed at the PFOA production plant.[14] Cross-sectional studies of PFOA-exposed workers at this plant have shown that PFOA was associated with decreased free testosterone and increased estradiol.[20]

To determine whether mortality from any cause was associated with occupational exposure to PFOA, a retrospective cohort mortality study was conducted at a plant that has produced PFOA since 1947.

Methods

The plant consists of several divisions, with PFOA production restricted to the Chemical Division. A number of other specialty chemicals have been produced in this division. The study cohort consisted of workers who were employed at the plant for at least 6 months between Jan 1, 1947, and Dec 31, 1983. Data were abstracted from plant personnel records, which were maintained on all workers ever employed at the plant. Vital status was ascertained from the Social Security Administration for the period 1947 to 1982 and from the National Death Index for the period 1979 to 1989. All workers with unknown vital status were traced using a variety of tracing strategies such as directory assistance, Metronet and TRW searches, reverse directories, motor vehicle registration lists, contacting neighbors and relatives, and the post offices. Death certificates were obtained from the appropriate state health departments for those identified as, or presumed to be, deceased. Information concerning the data and cause of two deaths which occurred outside the United States was obtained from family members. A nosologist coded the death certificates for underlying cause of death according to the International Classification of Diseases, 8th revision. The reliability of the coding was evaluated by resubmitting a random sample of death certificates for coding by the same nosologist. In the 25 death certificates from 1970 to 1989 resubmitted to the nosologist for ICD coding, there were no changes in the major categories of cause of death.

Workers were categorized as exposed or unexposed to PFOA based on their job histories. Exposed workers were defined as all workers employed for 1 month or more in the Chemical Division. Unexposed workers were employees who either never worked in the Chemical Division or worked in the Chemical Division for less than 1 month. Cumulative exposure to PFOA was estimated using the surrogate measure of months of Chemical Division employment.

The observed numbers of cause-specific deaths were compared to the expected numbers of deaths obtained by applying sex- and race-specific quinquennial age, calendar period, and cause-specific mortality rates for the United States and Minnesota populations to the distribution of observed person-time.[21,22] Because less than 1% of plant employees were nonwhite, white male and white female rates were used for comparison. For women, only United States rates were used because cause- and calendar period-specific Minnesota rates for women were not available. The effects of latency, duration of employment, and work in the Chemical Division were examined using stratified standardized mortality ratio (SMR) analyses. Cause-specific mortality rates were compared between exposed and unexposed workers using stratified SMRs.[23] SMRs were calculated for men based on US and Minnesota white male mortality rates for three latency intervals (10, 15, and 20 years) and three categories of duration of employment (5, 10, and 20 years). The SMRs were calculated using the program developed by Monson.[22]

The relative risk (RR) and 95% confidence interval (CI) for deaths from all causes, cancer, cardiovascular diseases, and other selected causes were estimated using proportional hazard models.[24,25] The time to event or censoring was defined as time from first employment to event or to December 31, 1989. In models for specific causes of death, deaths from other causes were censored at the time of death. Age at first employment, year of first employment, and duration of employment were included as covariates in the model. The analyses were stratified by gender. The appropriateness of the proportional hazard assumptions was tested using stratified models with graphical analysis of log (−log[survival]) versus follow-up time relationships and models that tested the significance of a product term between exposure and log follow-up time.[25,26] Proportional hazard calculations were conducted using SAS.[25]

Results

A total of 3537 workers employed at the plant between Jan 1, 1947 and Dec 31, 1983 were identified from company records. Six workers who had incomplete employment records were excluded from the study. The cohort consisted of 2788 (79%) men and 749 (21%) women (Table 1). Men

TABLE 1
Characteristics of Female and Male Employees, 1947–1989

	Chemical Division		Non-Chemical Division		Total	
	Female	Male	Female	Male	Female	Male
Number of workers	245	1339	504	1449	749	2788
Person-years of observation	6029.0	33385.3	13280.4	37732.4	19309.4	71117.7
Mean follow-up (y)	24.6	24.8	26.4	26.0	25.8	25.5
Mean age at employment (y)	28.8	25.6	26.9	28.9	27.6	27.3
Mean year of death	1965.0	1963.8	1962.8	1962.3	1963.5	1963.0
Mean year of death	1981.3	1978.3	1979.2	1978.1	1979.6	1978.2
Mean age at death (y)	58.7	54.2	54.4	58.1	55.4	56.4

contributed 71,117.7 person-years of observation, which were equally divided between the Chemical Division and non-Chemical Division. Women contributed 19,309.4 person-years, two-thirds of which were in the non-Chemical Division.

Vital status was obtained for 100% of the cohort (Table 2). There were 50 deaths among the women (11 in the Chemical Division cohort and 39 in the non-Chemical Division cohort) and 348 deaths among the men (148 deaths in the Chemical Division group and 200 in the non-Chemical Division group). Death certificates were obtained for 99.5% of deaths.

For women, the SMR for all causes of death (SMR = .75; 95% CI, .56 to .99) was significantly lower than expected (Table 3). There was no association with duration of employment or latency for deaths from all causes, cancer, and cardiovascular diseases (data not shown). Mortality among Chemical Division women was less than expected. In Chemical Division women, the all-causes SMR was .46 (95% CI, .23 to .86) and the cancer SMR was .36 (95% CI, .07 to 1.05). The all-causes SMR for the non-Chemical Division women was .91 (95% CI, .64 to 1.24) and the cancer SMR was .91 (95% CI, .49 to 1.52) (data not shown).

Using Minnesota rates for comparison, the SMR for men for all causes, for cardiovascular diseases, and for all gastrointestinal diseases was significantly less than 1 (Table 4). None of the cause-specific SMRs was large nor was any significantly different from 1. The results were similar when the expected numbers of male deaths were based on US mortality rates. For the three latency intervals, the SMRs for deaths from all causes ranged from .75 to .77. For all cancers, the SMRs ranged from 1.06 to 1.12 and were nonsignificant.

Among men, there was no association between any cause of death and duration of plant employment. The all-causes SMRs were .86 (95% CI, .72 to 1.01) for the Chemical Division group and .69 (95% CI, .59 to .79) for the non-Chemical Division group (data not shown). The SMRs for prostate cancer were 2.03 (95% CI, .55 to 4.59) in the Chemical Division group and .58 (95% CI, .07 to 2.09) in the non-Chemical Division cohort. In the Chemical Division group, there were 4 observed and 2 expected deaths from prostate cancer. There was no significant association between any cause of death and latency in either exposure group. For the Chemical Division cohort, the prostate cancer SMR was 1.61 (95% CI, .32 to 4.70) in the greater than 15-year latency group.

Table 5 presents the final proportional hazard model for all-causes, all-cancer, and prostate-cancer mortality among the 2788 male workers employed for more than 6 months. The estimated relative risk for all-cause mortality for a 1-year increase in age at first employment was 1.08 (95% CI, 1.07 to 1.09). Year of first employment and duration of employment were negatively associated with deaths from all causes. The risk associated with months employed in the Chemical Division was small and nonsignificant.

In the final prostate cancer mortality model, length of employment in the Chemical Division was positively and significantly associated with prostate cancer risk. The relative risk for a 1-year increase in Chemical Division employment time was 1.13 (95% CI, 1.01 to 1.27) For 10 years' employment in the Chemical Division, the relative risk was estimated to be 3.3 (95% CI, 1.02 to 10.6) compared with workers never employed in the Chemical Division. Age at first employment was positively associated with prostate cancer mortality. Length of time employed in the Chemical Division was not significantly related to mortality from lung cancer, gastrointestinal cancer, pancreatic cancer, or diabetes mellitus.

TABLE 2
Vital Status and Cause of Death Ascertainment among Female and Male Employees, 1947–1989

Vital Status	Chemical Division				Non-Chemical Division				Total			
	Female		Male		Female		Male		Female		Male	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Alive	234	95.3	1191	88.9	465	91.6	1249	86.2	699	93.3	2440	87.5
Dead	11	4.7	148	11.1	39	8.4	200	13.8	50	6.7	348	12.5
Total	245	100.0	1339	100.0	504	100.0	1449	100.0	749	100.0	2788	100.0

TABLE 3
Observed (Obs) and Expected (Exp) Deaths, Standardized Mortality Ratios (SMR) and 95% Confidence Intervals (CI) for 749 Female Employees

Cause of Death	Obs	Exp	SMR	95% CI
All causes	50	66.74	0.75	0.56–.99
Cancer	17	23.04	0.71	0.42–1.14
Gastrointestinal	2	4.54	0.44	0.05–1.59
Respiratory	4	4.72	0.95	0.26–2.43
Breast	3	5.87	0.51	0.10–1.49
Genital	2	3.37	0.59	0.07–2.14
Lymphopoietic	3	2.04	1.47	0.30–4.29
Cardiovascular	10	12.39	0.81	0.49–1.29
Cerebrovascular	3	3.51	0.86	0.01–4.80
Gastrointestinal	3	3.41	0.88	0.18–2.57
Injuries	4	6.23	0.64	0.17–1.64
Suicide	1	1.78	0.56	0.01–3.13

Discussion

This was the first retrospective cohort mortality study of workers employed in a PFOA production plant. Mortality from all causes in both men and women was significantly less than expected. Because of the healthy worker effect, internal comparisons

TABLE 4
Deaths and Standardized Mortality Ratios (SMR) Based on Minnesota White Male Rates, Among 2788 Male Employees, 1947–1989, and 1339 Men Ever Employed in the Chemical Division, 1947–1989

Causes of Death	All Male Employees				Men Employed in Chemical Division			
	Obs	Exp	SMR	95% CI	Obs	Exp	SMR	95% CI
All causes	347	450.79	0.77	0.69–0.86	148	172.96	0.86	0.72–1.01
Cancer	103	97.29	1.05	0.86–1.27	40	36.31	1.10	0.79–1.50
Gastrointestinal	24	26.78	0.90	0.57–1.33	9	9.77	0.92	0.42–1.75
Colon	9	9.42	0.96	0.44–1.81	4	3.46	1.15	0.31–4.01
Pancreas	8	5.58	1.43	0.62–2.83	4	2.04	1.96	0.53–5.01
Respiratory	31	30.42	1.02	0.69–1.45	12	11.26	1.07	0.55–1.86
Lung	29	28.94	1.00	0.67–1.44	11	10.70	1.03	0.51–1.84
Prostate	6	6.07	0.99	0.36–2.15	4	1.97	2.03	0.55–4.59
Testis	1	0.92	1.09	0.01–6.05	1	0.44	2.28	0.03–12.66
Bladder	3	2.18	1.37	0.28–4.01	1	0.75	1.33	0.02–7.40
Lymphopoietic	13	12.07	1.09	0.57–1.84	5	4.76	1.05	0.34–2.45
Cardiovascular	145	212.19	0.68	0.58–0.80	54	76.65	0.70	0.53–0.92
CHD*	110	159.09	0.69	0.57–0.83	43	57.74	0.74	0.54–1.00
Cerebrovascular	10	24.66	0.60	0.32–1.02	4	8.53	0.47	0.13–1.20
All gastrointestinal	12	21.13	0.57	0.29–0.99	8	8.27	0.97	0.42–1.91
All respiratory	13	21.75	0.60	0.32–1.06	7	7.77	0.91	0.36–1.87
Diabetes	8	6.52	1.23	0.53–2.42	3	2.55	1.18	0.24–3.44
Injuries	38	47.74	0.80	0.56–1.08	31	31.72	0.98	0.66–1.39
Suicide	12	15.09	0.79	0.41–1.39	10	6.99	1.43	0.68–2.63

*CHD, coronary and atherosclerotic heart disease.

were made between Chemical Division and non-Chemical Division employees. There were no significantly elevated SMRs in Chemical Division or non-Chemical Division employees. However, prostate cancer mortality was associated with length of employment in the Chemical Division in proportional hazard analysis. Ten years of employment in the Chemical Division was associated with an estimated 3.3-fold increase (95% CI, 1.02 to 10.60) in prostate cancer mortality.

The use of prostate cancer mortality to assess the association between PFOA and prostate cancer occurrence is problematic. Age-adjusted prostate cancer mortality rates from 1983 to 1989 (949 per 100,000) were only 25% of the incidence rates (99.4).[27] This low proportion of deaths among cases attributed to prostate cancer reflects the high risk of death for competing causes for this disease of elderly men. Given the small number of observed deaths from prostate cancer in the study, and the observed difference in incidence and mortality rates, the suggested association between PFOA exposure and prostate cancer must be viewed as hypothesis generating and should not be overinterpreted. The association may be real, may have been a chance finding, or may be the result of an unrecognized environmental factor. However, the biologic plausibility for any association between PFOA employment and prostate cancer is provided by animal toxicologic and human epidemiologic data that show an association between PFOA and reproductive hormone changes.[20]

The all-causes, all-cancer, and all-cardiovascular mortality among women was less than expected in the overall cohort. The low SMRs are most likely to be a result of the healthy worker effect. Latency and duration of plant employment did not have a strong relationship with the healthy worker effect.

The interpretation of this study requires consideration of methodological issues. SMRs for the subgroups of workers are not strictly comparable. We attempted to calculate standardized rate ratios; however, the rates were based on small numbers and produced unstable ratios. Estimates of PFOA exposure were based on job history, and categorization of workers into ever versus never employed in the Chemical Division may not reflect the biologic effective dose of PFOA. PFOA exposure was apparently widespread among employees not directly exposed to PFOA,[14] and the exposure categorization may misclassify workers as unexposed when they were ex-

TABLE 5
Proportional Hazard Regression Model of Factors Predicting Mortality among All Male Employees*

Variable	All Causes of Death				Cancer Deaths				Prostate Cancer Deaths			
	β	SE(β)	P	RR†	β	SE(β)	P	RR†	β	SE(β)	P	RR†
Year of first employment	−0.55	0.009	0.0001	0.946	−0.031	0.019	0.11	0.969	0.010	0.081	0.9	1.011
Age at first employment (y)	0.079	0.006	0.0001	1.08	0.078	0.011	0.0001	1.081	0.082	0.045	0.06	1.085
Duration of employment (y)	−0.34	0.001	0.0001	0.967	−0.028	0.009	0.002	0.972	−0.07	0.052	0.18	0.932
Months in chemical division	0.001	0.001	0.24	1.001	0.002	0.001	0.2	1.002	0.01	0.005	0.03	1.01

* Abbreviations used are: β, regression parameter; SE(β), standard error of the slope parameter; RR, relative risk.
† Relative risk for one unit change in independent variable.

posed. Such misclassification would be expected to bias the effect estimates toward the null if increased exposure increases death rates. Months employed in the Chemical Division may better reflect the biologic effective dose because cumulative exposure reflects the bioaccumulation of PFOA. Workers were exposed to many other xenobiotics, such as benzene and asbestos, during their employment at the plant. However, none of these materials has been associated with prostate cancer.

Although the mean age at first employment and mean year of first employment are similar in the Chemical Division and non-Chemical Division cohorts of men and women, the comparisons of the rates of disease are confounded by differences in the distribution of age at risk. The use of an internal comparison group may reduce, but not eliminate, confounding if the internal comparison groups have different distributions of these time factors. Because the disease occurrence relationship is defined in terms of cumulative exposure, the true effect of PFOA exposure may have been biased toward or away from the null by uncontrolled confounding by time factors.[28,29]

Further research is needed to evaluate and confirm the association between PFOA and prostate cancer. The findings in this study are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors. Studies of prostate cancer incidence in this and other PFOA-exposed work forces may clarify the suggested increase in prostate cancer risk.

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The Modulation of Rat Liver Carcinogenesis by Perfluorooctanoic Acid, a Peroxisome Proliferator

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The Modulation of Rat Liver Carcinogenesis by Perfluorooctanoic Acid, a Peroxisome Proliferator. ABDELLATIF, A. G., PRÉAT V., TAPER, H. S., AND ROBERFROID, M. (1991). *Toxicol. Appl. Pharmacol.* **111**, 530–537. Perfluorooctanoic acid (PFOA) is a peroxisome proliferator. The aim of this study was to test for its ability to act as a positive modulator of hepatocarcinogenesis, in the so-called biphasic (initiation by diethylnitrosamine 200 mg/kg ip followed by treatment with the suspected modulators) and triphasic (initiation by the same dose of diethylnitrosamine followed by a selection procedure for 2 weeks consisting of giving 2-acetylaminofluorene and in the middle of this treatment a single dose of CCl_4 followed by treatment with the suspected modulators) protocols of liver carcinogenesis. In both protocols treatment with PFOA increased the incidence of malignant hepatocellular carcinoma (HCC). As compared to phenobarbital, the modulating effect of PFOA is more pronounced in a biphasic than in the triphasic protocol. In parallel with positive modulation of HCC, PFOA also selectively induced the peroxisomal acyl-CoA oxidase activity and, to a lesser extent, catalase activity.

Perfluorinated fatty acids and their derivatives have many commercial uses due to their antiwetting and surfactant properties as well as their chemical and thermal stabilities (Guenthner and Victor, 1962). These compounds are used as water repellant, corrosion inhibitor, heat exchanger, and hydraulic fluid (Clark *et al.*, 1973). Generally, perfluorocarbons are considered metabolically inert and relatively nontoxic compounds (Sargent and Seffl, 1970). Recently, however, perfluorinated compounds such as perfluorooctanoic acid, perfluorodecanoic acid, and ammonium perfluorooctanoate have been shown to be peroxisome proliferators (Ikeda *et al.*, 1985; Kennedy, 1987; Pastoor *et al.*, 1987; Harrison *et al.*, 1988). Like the other peroxisome proliferators, hypolipidemic drugs (clofibrate, fenofibrate, and tibric acid), plasticizers (phtalate derivatives), and herbicides (phenoxyacetic acid derivatives), these compounds induce hepatomegaly, endoplasmic reticulum, and peroxisomal proliferation as well as peroxisomal metabolism of fatty acids (Moody and Reddy, 1978; Reddy and Lalwani, 1983; Ikeda *et al.*, 1985; Pastoor *et al.*, 1987; Harrison *et al.*, 1988). Many peroxisome proliferators are carcinogenic after long term administration to rodents (Reddy *et al.*, 1980; Reddy and Lalwani, 1983; Rao *et al.*, 1984, 1987). Some of these compounds have also been shown to be cancer promoting agents, mostly in rat liver (Reddy and Rao, 1978; Préat *et al.*, 1986). However, due to the lack of effect of these compounds on the incidence of γ-glutamyltranspeptidase positive hepatic foci which are often taken as a marker for evaluating such an activity, many of these studies have given contradictory results (Conway *et al.*, 1989).

We have recently proposed the term positive modulation (Roberfroid, 1987) to describe the effect of a supplementary treatment which

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The histological analyses of malignant tumors in the PB-treated rats indicated that 1 rat had type-IV HCC and 5 rats had type-I HCC, whereas the malignant tumors in the PFOA-treated rats were mainly of type-I HCC. In the PB-treated rats, many mainly eosinophilic and mixed cell type foci and nodules were observed, whereas in the PFOA-treated ones, some eosinophilic, basophilic, or mixed cell type and a few nodules were detected.

Peroxisomal Enzyme Activities

Treatment with PB never had any significant effect on the enzyme activities which were measured.

Catalase activity (Fig. 2). Both low (0.005%) and high (0.015 or 0.02%) doses of PFOA in diet induced catalase activity but this effect depended on both the dose and the duration of treatment. Rats previously submitted to DEN and DEN+2-AAF treatment were in general more susceptible to the catalase inducing effect of PFOA than control rats. In control rats (group A) the induction of catalase by PFOA was both dose and time dependent but it plateaued at 147%.

In DEN-treated rats (group B) the induction was always comparatively higher than in controls. It was still dose dependent if measured after 3 months of treatment, but it was time dependent only if the animals received the lowest but not the highest dose of PFOA. In rats submitted to the triphasic protocol (both I and S) the induction of catalase activity by PFOA (0.015% in diet) reached 250% after 7 months.

Acyl-CoA oxidase activity (Fig. 2). The induction of acyl-CoA oxidase activity by PFOA was much stronger than its effect on catalase, thus confirming a specific induction of the peroxisomal fatty acyl-CoA oxidase by peroxisome proliferators (Lazarow and de Duve, 1976; Lazarow, 1977; Pastoor *et al.*, 1987; Harrison *et al.*, 1988). At the lowest dose of PFOA (0.005% in the diet), the induction of acyl-CoA oxidase was not time dependent. Moreover, it was not influenced by pretreatment with DEN. Except at one time point (3 months), a result which had been repeatedly obtained, pretreatment with DEN did not in-

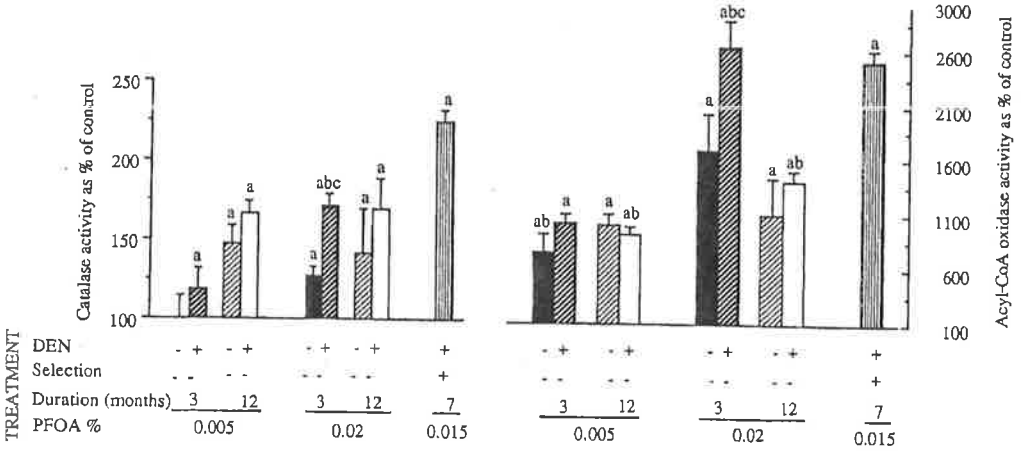

FIG. 2. Effect of chronic administration of PFOA to rats submitted to either a biphasic or a triphasic protocol for hepatocarcinogenesis on liver catalase and acyl-CoA oxidase activities. Statistical analysis indicated that values marked a are significantly different from respective control; b, which resulted from the effect of a high dose of PFOA (0.02%), are significantly different from the corresponding values of rats treated with the low dose of PFOA (0.005); c showed significant effect of initiation.

fluence acyl-CoA oxidase induction by 0.02% PFOA. Such an induction was, however, higher than that obtained using a lower dose of the PFOA.

In rats submitted to the triphasic protocol, the peroxisomal acyl-CoA oxidase activity was highly sensitive to PFOA and the induction of this enzyme was higher than that of catalase.

D-amino-acid oxidase and glycolate oxidases (Table 2). As opposed to catalase and acyl-CoA oxidase, the activity of the peroxisomal enzymes oxidizing D-amino acids and glycolate was not induced by PFOA treatment. The activity of D-amino-acid oxidase was reduced by such treatment but only after 3 months. Once again the effect of PFOA at both low and high doses was more pronounced if rats had previously been treated with DEN. Even though not presented here, the results were essentially the same in rats submitted to the triphasic protocol and subsequently treated with PFOA.

DISCUSSION

The study using both biphasic (initiation–promotion) and triphasic (initiation–selection–promotion) protocols demonstrates that PFOA, like other peroxisome proliferators, has a positive modulating activity on rat liver carcinogenesis. It must be emphasized that such treatment may also be a poor inducer of early neoplastic lesions at least in a two step protocol. In both protocols the malignant tumors in the PFOA-treated rats were HCC type-I and -III. PFOA is a stronger positive modulator of hepatocarcinogenesis than PB in the biphasic but not in the triphasic experimental model. Such an observation has also been reported for nafenopin as compared to PB (Préat *et al.*, 1986).

The mechanism of the positive modulation of carcinogenesis by peroxisome proliferators is not clear yet. All of these products share the property of inducing peroxisomal acyl-CoA oxidase activity (Moody and Reddy, 1978; Reddy and Lalwani, 1983) that may lead to an overproduction of H_2O_2 due to a limited induction of catalase activity that might create an imbalance between H_2O_2 production and degradation. The overproduced H_2O_2 may leak out of peroxisomes, induce DNA damage, or be transformed to other highly toxic products like superoxide anion and hydroxyl radical and which also have the ability to induce such DNA damage (Reddy *et al.*, 1982; Warren *et al.*, 1982; Reddy and Lalwani, 1983;

TABLE 2

D-AMINO-ACID AND GLYCOLATE OXIDASE ACTIVITIES IN RATS SUBMITTED TO THE BIPHASIC EXPERIMENTAL PROTOCOL

Enzyme	Duration of experiment (months)	Control rats (group A)			DEN-treated rats (group B)		
		Basal diet	0.005% PFOA	0.02% PFOA	Basal diet	0.005% PFOA	0.02% PFOA
D-Amino-acid oxidase	3	39 ± 4	32 ± 3*	23 ± 2*	29 ± 3	11 ± 1.9*,**	15 ± 0.8*,**
Glycolate oxidase	—	11 ± 1	15 ± 0.9	14 ± 2	9 ± 1	9 ± 0.7	12 ± 2
D-Amino-acid oxidase	12	38 ± 2	32 ± 4	32 ± 4	34 ± 4	28 ± 3	33 ± 3
Glycolate oxidase	—	10 ± 0.9	9 ± 0.6	7 ± 0.8	7 ± 0.4	7 ± 0.4	8 ± 0.7

Note. Enzyme activities were expressed as mU/mg of protein + SER.

* $p < 0.05$ vs basal diet.

** $p < 0.05$ vs group A (initiation has an effect).

Fahl *et al.*, 1984). All these reactive species may also participate in the initiation of lipid peroxidation (Goel *et al.*, 1986) and subsequent membrane destruction. Our results give support to the above-mentioned hypothesis. Treatment with PFOA induces the acyl-CoA oxidase by a factor of up to 26-fold, depending on the dose and duration of treatment as well as of the type of experimental protocol applied, whereas the induction of catalase activity by such treatment is always much lower, reaching a maximum of only 2.2-fold. However, this hypothesis has been challenged by several authors (Conway *et al.*, 1989). Moreover, other factors such as cell proliferation could also be involved.

It is worth emphasizing here that the induction of catalase activity by PFOA is very much influenced by previous treatment with DEN or DEN+2-AAF. Such a difference in sensitivity to the PFOA inducing effect is, however, less clear when measuring acyl-CoA oxidase activity. This may be due to the already very high level of induction appearing in control rats which makes it difficult to see any further induction. Such a difference between control and treated rats is, however, confirmed when measuring the reduction of D-amino-acid oxidase activity. At early stages during carcinogenesis (3 months) such a difference in the induction or inhibition of peroxisomal enzymes by PFOA treatment between control and initiated rats cannot be explained by proliferation of altered hepatic foci which often precede the appearance of malignant tumours. Indeed such lesions do not seem to develop significantly during PFOA treatment. This difference must thus be due to an increased sensitivity of initiated liver parenchyma to the effect of PFOA, an effect that requires further investigation.

Finally we can conclude that perfluorinated fatty acids need to be considered peroxisome proliferators having a positive modulating activity on liver carcinogenesis in rodents. Like the other peroxisome proliferators, this activity might be related to the drastic induction of peroxisomal β-oxidation. Additional work is in progress to provide more clarification about these effects.

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